ALGAL RESOURCE DEPRESSION BY MACRO-INVERTEBRATE HERBIVORES IN A CHALK STREAM: AN EMPIRICAL APPROACH

HELEN MARIE VINCENT

A thesis submitted to
The University of Birmingham
For the degree of
DOCTOR OF PHILOSOPHY

School of Geography, Earth and Environmental Sciences
The University of Birmingham
September 2008

ACKNOWLEDGEMENTS

This research was supported by a NERC studentship and co-funded by CEH.
I would like to thank Dr Mark E. Fudger, and Dr Sandy Milner for their supervision. Dr John Murphy also provided invaluable practical help and advice as my CEH case partner supervisor. Thanks to everybody at CEH Dorset, whose support was greatly appreciated, in particular my colleagues from the River Communities group including: François for fishing out all those length mass regressions; Rick Gunn, for all the identifying hell; Tracy Corbin for putting me up (& putting up with me); John Davy-Bowker for being very accommodating; Cynthia Davies for data organisation advice; John Blackburn for great ID advice; Nick Kneebone and Martin Neale for having a laugh in the lab; Mike Furse for writing me a great reference; Rebecca Harris for help in the field; and finally Patrick Armitage, who not only kept me motivated and inspired throughout, but also provided deeply appreciated moral support!
Further thanks go to the FBA, who provided excellent field facilities, and in particular Brian Godfrey who was most helpful. Also thanks to Mike Acreman for giving me time to write my thesis, whilst working for CEH.
Thanks to my family; my Mum Linda; Brother Martin and his girl friend Lizzy; Dad Michael and Val, and Granny and Grampy, for individual help and support over the years! Many friends supported me during this thesis including: Sophie Eldridge, Beth Haines, Katharine York, Claire Cailes, Gemma Wilson, Cat Muller, Cath Jex, Heather Thompson and Helen Walker. Thanks to staff at the University of Birmingham: Andy Baker, Richard Johnson and Gretchel Coldicott.
Finally I would like to thank François mostly for his love and steadfast support throughout, particularly the last few weeks, when the crumbs started to take over and he taught me to say……hell to the crumbs - life is too short…… let's make babies, and along came Skye! But also I want to say thanks for all the practical help and support François gave during the final weeks and during the correction process, without which I'd probably have gone nuts!!

ABSTRACT

Herbivory is a globally important ecosystem function, occurring in all major biome types; including benthic freshwater habitats. Algal biofilms and their herbivore consumers are therefore important components of stream food webs. However there is relatively little empirical data quantifying the strength of these algal-herbivore interactions, or how these vary with herbivore identity, size, and biofilm physiognomy. Interactions across a diverse herbivore guild were investigated in a chalk stream, using mesocosms to determine the distribution of algal-herbivore interaction strengths. A series of experiments were used to assess: herbivore link strength distribution; context-dependency of interaction strength; the relationship of body size with interaction strength; and the effects of competing grazer species on algal resources. The algal-herbivore sub-web was dominated by weak interactions which concurred with empirical and theoretical evidence, and further supporting web stability theory. Interactions were highly context-dependent, with interaction magnitude and species identity both affected by algal biofilm type. Grazer species identity was important for determining body size relationships. Although competitive effects were apparent, they were not statistically detectable. This research builds on previous investigations of algal grazer interactions and food web structure by emphasizing; 1) the importance of grazing as an ecosystem function, and 2) the diversity of interactions occurring in model systems. The use of experimental mesocosms may be limited in terms of 'real' systems, but provide a valid response of model systems that are both useful and valid tools for assessing community ecology.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 – Introduction

1.1 Summary

This chapter describes the fundamental characteristics of herbivory in a global context, focusing on freshwater algal biofilms and macro-invertebrate grazers. The literature on grazer-algae interactions is reviewed, as well as the limitations of field and laboratory studies. Factors affecting the strength of grazer-algae interactions are described. Interaction strength is explained in the context of food webs. Research aims and predictions are outlined.

1.2 Introduction and background

1.2.1 *Herbivory: A global perspective*

Through the process of photosynthesis primary producers harness the energy of solar radiation to convert carbon dioxide and water into gaseous oxygen, and the building blocks of organic life: hydrocarbon molecules (Taylor et al, 1997). Although converting energy into biomass is not exclusively reliant on photosynthesis, (thermal vents, etc), it is without doubt the dominant mechanism (Begon et al., 1996). Once harnessed by photosynthesis, energy passes through biological systems from primary producers to primary consumers, secondary then tertiary consumers and beyond. With each progressive trophic level more and more energy is lost through heat, waste products and death (Begon et al., 1996). Consequently, the process of energy transfer between plant resources and primary consumers (herbivory), will govern energy available for higher trophic levels. Herbivory occurs wherever plants and their consumers exist; in terrestrial, marine and freshwater habitats across the planet. Herbivory in terrestrial systems is centred on vascular plants. Conversely, marine and freshwater systems are dominated by macro-algae and micro-algae respectively, with few vascular plants in these biomes. The result of herbivory across biomes appears to be similar for non-vascular plants; where reductions in biomass are comparable regardless of biome type, while effects on vascular plant biomass appears greater for marine systems (Lodge et al., 1998). However, evaluation of herbivore impacts across biomes should be done cautiously as divergence in plant taxonomy will, to some degree confound direct comparisons (Lodge et al., 1998).

Not only taxonomic differences exist. Terrestrial vascular plants and marine macro-algae tend to have longer generation times and larger body size than the equivalent

freshwater microalgae. The former have invested in greater evolutionary defences against herbivore consumption, such as cellulose and lignin which offer structural defence against grazing; and chemical defence compounds which reduce palpability to grazers (Lodge et al., 1998). Furthermore, large terrestrial plants and marine macro-algae are usually much larger than the herbivores. Consequently, these plants may sustain significant herbivore damage, yet survive attacks (Strong, 1992). Freshwater microalgae are much smaller and more susceptible to herbivory but compensate through short life cycles (days in some cases) and high turn-over rates (Feminella and Hawkins, 1995). Some pelagic freshwater algae do however, exhibit structural defences against herbivory through cell size and shape, or by forming colonies that act as size refugia (Agrawal, 1998; Yoshida et al., 2004). This strategy is effective in pelagic communities because phytoplankton and their zooplankton consumers are similar in size. Benthic algae in rivers and streams however, are by comparison over an order of magnitude smaller than the macro-invertebrates consuming them. Consequently, colonies of algal cells are readily consumed by herbivorous invertebrates (personal observation). Lentic primary production can also benefit from trophic cascades (Strong, 1992; Agrawal, 1998), where control of herbivores by secondary and tertiary consumers releases algae (pelagic phytoplankton, benthic lake littoral and epiphyte algal communities) from herbivory (Strong, 1992; Agrawal, 1998). Benthic micro-algae of river and stream systems on the other hand, benefit from upstream recruitment that counteracts the impacts of herbivory.

Of the three biomes described, freshwater systems have only recently attracted more interest (Lodge et al., 1998). Early models of herbivory in pelagic lake communities analysed simple impacts of zooplankton on the phytoplankton. Since then, detailed algal-herbivore interactions have been investigated in pelagic lake communities, and

benthic communities from both lakes and river systems (Feminella and Hawkins, 1995). With current research dedicated to developing detailed ecological models, the importance of mapping communities and network pathways has increased (Ings et al., 2009). Although community form and function from lake systems has received most investigation, rivers and streams are now starting to achieve a similar level of investigation (Ledger et al., 2006, 2008). A comprehensive understanding of the dynamics driving ecosystem form and function in our rivers and streams is essential for building the ecological models used by water resource managers, and where better to begin than at the bottom: autotrophs and their consumers.

1.2.2 *Benthic algae: A spotlight on lotic primary producers*

The benthic surfaces in the photic zone at the bottom of streams and rivers usually support micro-algal communities (Bott, 1983; Allan, 1995). Traditionally these algal biofilms were regarded as poor sources of autochthonous carbon in streams despite their widespread distribution (Lamberti, 1996). However, since a seminal paper by Minshall (1978), research over the past three decades (reviews in Wetzel, 1983; Feminella and Hawkins, 1995; Steinman, 1996) clearly demonstrates their importance as a basal resource for consumers (Rosemond et al., 1993).

The algal species composition and structure of the biofilm varies spatially depending on the nature of the surfaces they colonise. Although epilithic (stone surfaces), epipelic (soft sediments) and epiphytic (higher plant surfaces) biofilms all support ubiquitous species they nevertheless often develop characteristic assemblages specific to particular substrate types (Biggs, 1996). This is due to both the suitability of the substrate for attachment (Burkholder, 1996) and the fact that substrate type is also

closely associated with wider hydrological variables such as flow and depth (Bunte and Abt, 2001). Because of this, two of the most fundamental characteristics of substrate as habitat for algae are its particle size and its stability (Peterson, 1996). Thus, algal assemblages vary greatly both within and between stream and river systems, with changes in the morphology of the benthic habitat (Biggs, 1996).

From initial colonists to mature assemblages, biofilms follow distinct successional patterns (Tuchman and Stevenson, 1991). Succession in biofilms is driven by interspecific variation in colonisation, algal life history traits and competitive abilities of component species (Steinman, 1996). High discharge events dislodge algae, creating bare patches, and effectively reset community development (Peterson, 1996; Biggs and Smith, 2002). Initial attachment of unicellular, motile and less competitive algal species is succeeded by rapidly reproducing strong competitors that form multicellular filaments and tufts (Tuchman and Stevenson, 1991). Thus, biofilm physiognomy (three-dimensional structure, Whittaker, 1975) changes with the successional age of the assemblage, as does the richness and relative abundance of component species (Johnson et al., 1997). For example, early stage biofilms are species poor, dominated by tightly adhering cells, which give the biofilm a compact structure compared to mature communities with thicker biofilms composed of large, vertical cells and filaments (McCormick and Stevenson, 1991).

Two fundamental abiotic factors, light intensity and nutrient supply, influence the assemblage structure, turnover rate and biomass accrual of the algal biofilm (Borchardt, 1996; Hill, 1996). Light limited biofilms are of low biomass, dominated by small adnate diatoms and lack large overstory structures (Sumner and McIntire, 1982). By comparison, unrestricted light promotes the formation of thick biofilms comprised of large filamentous species, with associated diatoms, bacteria and

particulate matter (Johnson et al., 1997). However, attenuation of light in thick biofilms may have negative impacts for species close to the substrate surface (Tuchman and Stevens, 1991), and these become light-limited until the thickness of the biofilm is 'reset' e.g. due to disturbance (Biggs and Smith, 2002). Likewise, though overall nutrient concentrations are system specific and determine the potential biomass of algal biofilms, the thickness of the biofilm regulates the flow of nutrients to the basal layers (by absorption and modification of water flow, Dodds and Biggs, 2002), and these may become nutrient limited until the upper layers are removed (Biggs and Smith, 2002). For example, Sumner and McIntire (1982) found artificial nutrient enrichment caused eutrophication of the biofilm assemblage allowing dominant species to bloom, producing a decline in species richness.

In summary, physical disturbance, substrate type, light incidence and nutrient supply all contribute to the formation of complex algal biofilms covering the benthic surface of streams and rivers. Hence, it is not surprising that many benthic animals are adapted to exploit this abundant resource (Cummins and Klug, 1979), and that through their feeding, algae form a pivotal role in of lotic food webs. A closer examination of these animals is therefore required.

1.2.3 *Benthic invertebrates: The consumers that transfer autotrophic energy into secondary production*

Macro-invertebrates span a variety of trophic levels within freshwater food webs, but many are herbivores and feed on benthic algal biofilms (Allan, 1995). Herbivores perform an important function in the stream benthos, essentially transferring energy captured in primary production to secondary consumers (Rosi-Marshall and Wallace,

2002). Macro-invertebrate herbivores can be assigned to functional feeding groups (FFG, Cummins, 1973) depending on their mouthpart morphology (e.g., grazers, collector-gatherers, and filterers). Filterers remove suspended particles from the water column, using either silk nets, spun to form traps (some caseless caddisflies) or possess morphological adaptations, such as the cephalic fans of blackfly larvae (Cummins and Klug, 1979; Allan, 1995). Collector-gatherers primarily use several sets of setae to comb fine particulate organic matter (FPOM) from the loose material on the substratum (Allan, 1995; Diaz Villanueva and Albarino, 2003). Grazers remove attached algae from the benthos (Allan, 1995; Steinman, 1996). However, within the grazer FFG considerable variation in mouthpart structure exists and can lead to different modes of feeding. For example, animals can rasp (gastropods), browse (mayfly nymphs) and scrape (caddisfly larvae) algae from the substratum (Lamberti et al., 1987; Hill and Knight, 1988). Different morphologies for removing and ingesting algal cells ensure maximum algal assimilation, and in effect aid resource partitioning by exploitation of different resources via the different morphologies (Steinman, 1996; Tall et al., 2006).

Biofilm susceptibility to grazing depends on the compatibility between physiognomy and grazer mouthparts (Sumner and McIntire, 1982) For example; the radula of gastropod molluscs efficiently removes closely adhering algae, whereas the brush-like mandibles of mayfly larvae browse loose, upright algal forms (Sumner and McIntire, 1982; Lamberti et al., 1987; Hill and Knight, 1988). Consequently, the interaction between grazers and algae is to some degree influenced by grazer morphology and biofilm structure, the latter perhaps having received less attention than the former in grazing experiments (Feminella and Hawkins, 1995).

1.2.4 *Studies of grazer-algae interactions: how do benthic algae and invertebrate consumer interact?*

Negative impacts of grazers on algal biofilms

In the past ecologists have used a variety of empirical methods to assess grazer effects, such as exclusion of target species in natural systems (Lamberti and Resh, 1983; Kohler, 1992; Jordan and Lake, 1996; Peterson et al., 1998; Barbee, 2005), removal of target species from natural systems (Brown et al., 2000; Taylor et al., 2002; McNeely et al., 2007), enclosure of target species in mesocosms (Sumner and McIntire, 1982; Lamberti et al., 1987; Steinman et al., 1987; DeNicola and McIntire, 1991; Alvarez and Peckarsky, 2005) and enclosure of target species within stream-side channels (Hill and Knight, 1988; Tuchman and Stevenson, 1991; Rosemond et al., 1993; Rosenfeld, 1997; Ledger et al., 2006, 2008).

Evidence of strong negative grazer impacts on algal biomass for a number of widespread grazing taxa dominates the literature (reviews in Cattaneo and Mousseau, 1995; Feminella and Hawkins, 1995; Hillebrand, 2008). For example, studies assessing caddisfly larvae, gastropod molluscs and mayfly larvae all report significant grazer effects on algal biomass (Sumner and McIntire, 1982; Lamberti et al., 1987; Steinman et al., 1987; Hill and Knight, 1988; Lamberti et al., 1989; Bronmark et al., 1991; DeNicola and McIntire, 1991; Peterson et al., 1998; Chase et al., 2001; King-Lotufo et al., 2002; Alvarez and Peckarsky, 2005; Holomuzki and Biggs, 2006).

These studies conclude that the two causes of algal biomass reduction by grazers are consumption of algae (a direct trophic effect) and dislodgement of the biofilm when foraging (a direct non-trophic effect), (Liess and Hillebrand, 2004). It is difficult to

separate consumptive biomass reduction from grazer dislodgement and subsequent export (Hill and Knight, 1988; Lamberti et al., 1989). Big and bulky animals moving across the substrate such as gastropod molluscs and cased caddisflies, which cut through the biofilm on large muscular feet and drag heavy stony cases respectively, export more algae than light and agile counterparts (Lamberti et al., 1987; Poff et al., 2003). Long algal tufts or filaments anchored by dead or senescent basal structures may slough more readily by invertebrate dislodgement compared to biofilms composed of tightly bound adnate forms (Hill and Knight, 1987). Therefore, the interaction between biofilm physiognomy and grazer morphology has implications for downstream export as well as immediate local consumption (Rosemond et al., 1993).

Although most studies report that overall strong grazer / algal interactions usually lead to reduced algal biofilm biomass and productivity (Steinman, 1996), grazer specific differences in resource depression occur and may have consequences for the presence and abundance of algal taxa in biofilms, affecting the species composition and physiognomy of the biofilm (Diaz Villanueva and Albarino, 2003). Steinman (1996) concludes that different levels of grazing pressure have different effects on algal assemblage structure. Predominantly, low and intermediate levels of grazing effect algal diversity very little, but intense grazing pressure reduces diversity (Underwood and Thomas, 1990). In contrast, Tuchman and Stevenson (1991) found ambient densities of grazing snails promote algal diversity. They attributed this to an increase in evenness caused by grazer removal of numerically dominant overstory species. Even if changes in richness and evenness appear to remain constant over the duration of an experiment, fluctuations in species composition can still occur (Liess and Kahlert, 2007).

Positive impacts of grazers on algal biofilms

Conversely, some studies report increased algal biomass as a result of grazing, though they are less abundant in the literature (Steinman, 1996). They tentatively suggest nutrient regeneration as a possible cause (reviewed by Feminella and Hawkins, 1995). For example, increased nutrient input from grazer faecal material may promote algal biomass accrual in nutrient poor habitats, as primary production and consumption will be low (Wotton and Malmqvist, 2001). In contrast, nutrient regeneration effects are likely to be negligible in highly productive systems where light and nutrient limitation is not relevant (Feminella and Hawkins, 1995). Furthermore, grazing can also trigger increased primary production via the removal of dead and senescent cells that restrict light and nutrients to viable cells (Lamberti and Resh, 1983; Lamberti et al., 1989; McCormick, 1994; Steinman, 1996; Liess and Hillebrand, 2004). Consequently, grazing can stimulate high turnover rates in benthic biofilms. Therefore, comparatively low primary producer biomass is able to support large numbers of grazers (Lamberti and Resh, 1983).

No impact of grazers on algal biofilms

Finally, some studies show no observable grazer reduction in algal biomass, and Steinman (1996) speculates that low grazer densities, inappropriate mouthpart morphologies and poor initial algal accrual (caused by resource limitation) are possible causes. For example, Steinman et al (1987) found a threshold snail density was necessary to drive a reduction in algal biomass; Diaz Villanueva and Albarino

(2003) attributed low mayfly consumption to a mis-match between mouthpart morphology and algal biofilm structure; Feminella et al (1989) identified very low grazer impacts to extremely low inorganic nitrogen levels.

1.2.5 S*trength of grazer-algae interactions*

Influence of grazer traits and biofilm physiognomy

Although interactions are consistently detected, the magnitude of effect exerted by grazers on algal assemblage structure, physiognomy and biomass varies between studies (Steinman, 1996). This has been attributed to differences in 1) grazer taxon characteristics, such as mouthpart morphology and feeding mode (Sumner and McIntire, 1982; Hill and Knight, 1988); and 2) algal properties, such as position in the biofilm and digestibility (Lamberti et al., 1989; Peterson et al., 1998; Peterson and Jones, 2003).

Predictions of herbivore efficiency and of impacts on algal community structure can be inferred by considering the mouthpart morphology of target grazer species. For example, Hill and Knight (1988) found that different species, with different morphologies, yielded different magnitudes of effect on algal biofilms. *Neophylax* spp. (Uenoidae) caddisfly grazers greatly reduced all algal constituents, whereas *Ameletus* spp. (Ameletidae) mayfly larvae only reduced loose upper layers of algal biofilms. Similarly, Lamberti et al (1987) found that *Dicosmoecus gilvipes* (Limnephilidae), a caddisfly with scraping mandibles efficiently reduced algal biomass (AFDM and chlorophyll *a*) and altered community structure, producing a homogeneous monolayer. In contrast snails (*Juga silicula*, Pleuroceridae) with

rasping teeth and mayflies (*Centroptilum elsa*, Baetidae) with delicate brush-like mandibles had a reduced effect on the overstory component of biofilms. Thus the strengths of these interactions were determined by interplay between the characteristics of the grazer and of the algae.

Additionally, differential ingestion and digestion of algal cells by grazers have implications for biofilm biomass regulation (Peterson et al., 1998; Diaz Villanueva and Albarino, 2003). Overstory algal cells, susceptible to grazer ingestion as a consequence of their position in the biofilm, tend to be resistant to digestion (Peterson et al., 1998). However, as resistant algal cells pass through the gut they exude exogenous organic substances, which provide grazers with an alternative form of nutrition (Underwood and Thomas, 1990; Diaz Villanueva and Albarino, 2003). Conversely, tightly adhering adnate cells, which are less likely to be removed by grazers, are more susceptible to digestion (Peterson et al., 1998). The strength of the grazer-algae interaction thus becomes difficult to interpret if an algal cell is consumed but remains viable once excreted.

Assessing algal responses in terms of mouthpart morphology and biofilm physiognomy alone does not take into account differences between grazing rates. The locomotion mode, for example semi sedentary or highly mobile, is also a determinant of the impacts of grazers on algal biofilms. The success of *D. gilvipes* at reducing algal biofilms was not only attributed to efficient mandible action (Lamberti et al., 1987), but also as a result of a high consumption rate as it moved over the substrate rapidly (Steinman, 1996). However grazing rate is not solely determined by grazer identity. King-Lotufo et al (2002) found that grazing rates increased with grazer density, but also found that grazing rates increased with algal biomass.

The influence of ontogeny: individuals and populations

As a grazer species progresses through its life cycle it will undergo morphological, physiological and behavioural changes that ultimately have implications for its ability to crop and assimilate algal cells (Allan,1995). Many predatory invertebrate species, such as stoneflies and caddisflies, can be herbivorous at early instars (Siegfried and Knight, 1976; Baekken, 1981; Cereghino, 2002). Even species classified as grazers may switch from a mainly detritivorous diet to herbivory after the first or second instar (Baekken, 1981; Allan, 1995). Thus it cannot be assumed that the occurrence and strength of a grazer-algae interaction remains the same over developmental time.

Small grazers are limited by the size of their mouthparts and cannot exploit large resources, but as they grow the size range of prey items expands. As an individual develops it undergoes a related increase in metabolic requirements (Cohen et al., 1993). Alverson and Courtney (2002) associated a variation of grazing intensity with a shift in energy requirement for a particular life history stage of a grazing dipteran larva. They found that the 4th instar of *Blepharicera* (Blephaceridae) initially had low grazing intensity, which increased until pupation was imminent; mid-way through the instar. Therefore, the strength of the interaction between grazer and algae may well vary with ontogeny as nutritional requirements, behaviour and body size change, and the size and quantity of prey consumed increases.

These ontogenetic changes are also reflected at the population level, as cohorts of grazers develop their relative per capita interaction strength will ultimately change. Consequently, in early development a cohort may be constituted of individuals with low grazing rates, but the high densities of these populations of small individuals could have significant effects on algal biofilms (Sala and Graham, 2002). Conversely,

older populations that have lower densities (due to mortalities) will produce large individuals with higher per capita effects, but overall population-level impacts may be low. However, a population may experience density fluctuations, particularly if a population produces more than one cohort per annum. King-Lotufo et al (2002) suggest that at high population densities algal biomass is reduced, but that grazing rates are eventually depressed by density-dependent effects, such as interference competition and emigration.

The influence of competition and predation

At the community level, different types of grazers compete for shared algal resources and their combined effect on the biofilm can be complex. Hill and Knight (1988) and Poff et al (2003) found an additive effect of multiple grazers on algal responses which indicates that there was no facilitation or interference between the grazer species. On the other hand many studies have showed strong interference between grazers, within species (e.g. Gresens, 1995) and across morphologically similar (e.g. Chase et al., 2001) and morphologically different species (e.g. Kohler, 1992). How the impacts of different grazers combine reflects the overlap in their feeding niche i.e. the overlap in their diet and their feeding mode (Becker, 1990; Rosenfeld 2002). Additionally, these complex interactions are further modified in the presence of predators, due to the relative ability of different grazers to avoid predation (Lima and Dill, 1990; Tikkanen et al., 1994). The presence of predators may reduce the impact of a grazer by reducing its abundance (Peckarsky and McIntosh, 1998) or inhibiting its foraging (Culp and Scrimgeour, 1993; McIntosh and Townsend, 1996), but this may cause the competitive release of another grazer, less susceptible to predation (Holt, 1977). More

empirical studies are required to assess the potential interactions between different grazer species, and between grazer and non-grazer species, and their combined effects on algal biofilms.

Abiotic influences

The strength of grazer algae interactions may also be affected by natural abiotic fluctuations which influence the properties of the algal biofilm. The effects of varying light, flow and nutrient levels on grazer-algae interactions are well studied (Steinman, 1996), but are context and species dependant. For example, Lamberti et al (1989) detected strong effects of *Juga silicula* (Pleuroceridae) snails on algal biofilms (90% removal) at low light intensity because algal production was low and snails were food limited. This compared to less than 15% biofilm removal at high irradiance, when algal production was high and snails were not food limited. On the other hand, Sumner and McIntire (1982) found little difference in biofilm biomass in algal patches ungrazed or grazed by *Juga plicifera* (Pleuroceridae) at different light intensities, similarly to Liess and Kahlert (2007) with the snail *Viviparus viviparus* (Viviparidae). With respect to flow, Poff et al (2003) found grazing effects of the mayflies *Baetis bicaudatus* (Baetidae) and *Drunella grandis* (Ephemerellidae) and the caddisfly *Glossosoma verdona* (Glossosomatidae) varied under different experimental water velocities. At fast current, algal biofilm removal was equivalent among the species. At medium current, *D. grandis* removed significantly more than both *B. bicaudatus* and *G. verdona*, whereas at slow current, *D. grandis* removed more than *B. bicaudatus*, which removed more than *G. verdona*. With respect to nutrients, Liess and Kahlert (2007) showed that grazer-algae interaction strength was weaker at high

nutrient levels, because algal growth rates exceeded grazer consumption rates. On the other hand Sumner and McIntire (1982) found a higher impact of grazing at higher nutrient levels, but this was in part due to a shift in the algal assemblage with nutrient addition towards more easily grazed algal species. Additionally, Steinman (1996) highlights a measurement effect of nutrient limitation on the algal/grazer interaction, suggesting that, at low nutrient levels, reduced algal growth makes detection of grazer effects problematic in short term experiments. Interactions between biotic and abiotic factors are complex, and the importance of assessing herbivore / grazer interactions in the wider ecological context is clear.

1.2.6 *Grazer-herbivore interactions in food webs*

The diverse array of species in ecosystems can be viewed as a network of interacting species, in Darwin's words (1859, p. 59): plants and animals '*are bound together by a web of complex relations*'. Elton (1927) first proposed the concept of a food web, or network of species interacting via their feeding relationships. Grazer-algae interactions in freshwater ecosystems typify the animal-plant interface, where primary production enters the food chain via consumption and is incorporated into secondary production (Allan, 1995; Lamberti, 1996).

More recently, research has focused on the strength of individual feeding links, and how interaction strength is distributed in food webs (Berlow et al., 1999, 2004). Ecological theory predicts that community stability, i.e. the ability of a community to persist in the face of environmental change (e.g. pollution, global warming etc) and disturbance (e.g. floods etc), depends on the pattern and strength of interactions between species (Yodzis, 1981). The premise is that strong interactions destabilise

communities (de Ruiter et al., 1995; Neutel et al., 2002), because the loss of the strong interactors can set in motion changes in resource abundance and species distributions which may have unpredictable secondary effects, for example habitat modifications or secondary extinctions (Emmerson et al., 2004). It has been suggested that weak interactions in food webs stabilise the community (McCann et al., 1998), in the same way as omnivory, by dampening the relative influence of strong consumer-resource interactions (Berlow, 1999; McCann, 2000).

Nonetheless, much of this theory is derived from conceptual modelling (e.g. Laska and Wootton, 1998; Quince et al., 2005; Wootton and Emmerson, 2005; Karlsson et al., 2007) and it is unclear if speciose natural food webs do operate in this way (Abrams, 2001). Though there has been a drive to better understand the distribution of interaction strength in real food webs, much of these studies infer interaction strength from meta-analyses of existing data sets (e.g. Osenberg et al., 1999; Brose et al., 2006), and there is a need for more empirical research. In particular most studies focus on predator prey interactions (e.g. Wootton, 1997; Chalcraft and Resetarits, 2003), despite the fact that primary consumers and their plant resources are often the most abundant in ecological communities (Lindeman, 1942). Furthermore, most studies have been based in marine and terrestrial habitats (e.g. Paine, 1992; Menge et al., 1994, 1999), and detailed studies that attempt to measure interaction strength in freshwater systems are few.

1.2.7 *Limitations of grazer-algae studies*

Methodological aspects

Characteristics of algae and their grazing consumers are not the sole factors contributing to patterns in grazer-algae interactions. Experimental design, field or laboratory based, contributes to the overall effect. Feminella and Hawkins (1995) reviewed some of the differences in grazer effects between field and laboratory studies, and between long (> 4 months) and short (< 4 months) term studies. The results of their meta-analysis of contemporary literature found that long term laboratory studies produced greater grazer effects than shorter duration field studies. Other confounding effects include the nature of grazer exclusion/inclusions. Lamberti and Resh (1983) and Kohler (1992) reported increased algal biomass on elevated tiles, which effectively excluded the cased caddisflies *Heliopsyche* sp. (Helicopsychidae) and *Glossosoma* sp. (Glossosomatidae) respectively. By comparison, tiles available for caddisfly colonisation (placed on the streambed) showed signs of substantial algal biomass reduction. In fact, such designs may not be suitable across habitats, as it effectively excludes other grazer species less able to colonise elevated tiles. Thus, Feminella and Hawkins (1995) advise caution when extrapolating results from laboratory studies to natural systems. They also made the point that studies in natural systems are often carried out in spring and summer, when algae and grazers are at their most abundant, and that observed patterns may not be applicable all year round.

Geographical aspects

Geographical location also influences the impact of grazers on algae across studies (Feminella and Hawkins, 1995). Temperate regions and their associated macro-invertebrate grazer assemblages have been studied to a greater extent than neotropical or subtropical areas. Perhaps this is a result of a greater concentration of freshwater ecologists in temperate locations. Alternatively, this may reflect a greater diversity of algivorous fish (Flecker, 1992) and large decapods (Barbee, 2005) in tropical regions, which means small macro-invertebrates are not the dominant grazers and are not the focus of study. Furthermore, by exerting strong direct consumptive effects on biofilms, these very large grazers effectively mask any macro-invertebrate impacts. However, Barbee (2005) investigated tropical streams with few fish herbivores and found algal biomass decreased in a pattern similar to temperate experiments, suggesting that in this particular system macro-invertebrate grazers did have significant effects on biofilms.

Choice of target species

A further confounding aspect is that many empirical studies on the impact of herbivores use target species that traditionally are thought of as efficient grazers (particularly snails, caddisflies and mayflies), and species thought of as weak grazers are usually neglected, thus biasing results towards strong negative impacts on algal biofilms (Steinman, 1996). Also, little information is available on the impacts of species usually classified in other functional feeding groups, such as predators and shredders. These animals may rely on herbivory at different stages of their life

histories (Siegfried and Knight, 1976; Cereghino, 2002) or when availability of their primary food is reduced (Moore, 1975; Malmqvist et al., 1991; Edwards, 2005; Lancaster et al., 2005).

Single vs. multiple responses

Lamberti et al (1987) stressed the importance of assessing more than one biofilm response to grazing invertebrates. They attributed differences in grazer impacts across studies to a difference in the response variables measured. For instance, early grazer studies concentrated on measuring biomass and productivity, but neglected algal physiognomy or assemblage composition (Minshall, 1978).

Consequently, information may be lost by measuring single algal responses. On its own, algal biomass reduction may indicate a detrimental effect to biofilms. However, the indirect positive effect of the removal of the senescent biofilm layer by grazers on viable algal cells (via enhancement of light penetration and nutrient acquisition, Borchardt, 1996; Hill, 1996) may be greater than the direct negative effects of consumption (McCormick and Stevenson, 1991). These effects are only detectable if productivity is measured in conjunction with biomass. Thus, King-Lotufo et al (2002) found total biomass failed to detect grazer impacts to the same degree as chlorophyll *a* concentration. Quantification of multiple algal responses (AFDM, Chlorophyll *a*, biofilm physiognomy and species composition) to grazing pressure clarifies the underlying mechanisms, and can help explain how the strength of the grazer-algae interaction varies (Lamberti et al., 1987).

1.3 Research aims

The research presented in this thesis explores some of the factors governing the pattern and strength of interactions between benthic microalgae and herbivorous macro-invertebrates in chalk streams. These systems were chosen for this study because 1) they have a diverse macro-invertebrate assemblage, providing a spectrum of functional feeding groups within the herbivore guild (Ladle and Bass, 1981; Armitage et al., 2003); and 2) benthic primary production is high, thus algae are important in the food web dynamics (Marker, 1976 a,b). Furthermore, they are fairly temporally stable in terms of flow, temperature and nutrient loading, thus they provide ideal study systems (Hellawell et al., 1974; Sear et al., 1999; Bowes e al., 2005).

Interaction strength between grazers and algae was assessed using a standardised Dynamic Index (Wootton, 1997; Berlow et al., 1999), which permits comparison of interactions across experiments, species and systems. The variables measured included, at the community level, biofilm biomass, chlorophyll *a* concentration and total algal cell numbers; and at the population level cell numbers of individual algal species/species groups.

The following key questions were addressed:

1 – How are interactions strength distributed in the guild of herbivores? (Chapter 2)

Because existing studies have focused on selected key taxa, the strength of interactions was studied in mesocosm experiments across a wide range of herbivores, including several functional feeding groups, and including taxa that are not usually considered to be preponderantly herbivorous. Per capita and population level effects are derived using experimental and survey data. A guild of herbivores is expected to exert many weak effects and a few strong effects.

2 – Is the strength of grazer-algal interactions context dependent? (Chapter 3)

Few studies have measured the interaction strength of the same grazers across different resource types, thus mesocosms were used to test whether selected grazers had the same impact on two types of biofilm structures: thin crustose biofilms and thicker diatomous mat biofilms. Algal mats were expected to be affected more than algal crusts by grazing invertebrates.

3 – Does interaction strength change with body size? (Chapter 4)

Though some studies indicate interaction strength increases with body size, they are biased towards engulfing predators, one to three orders of magnitude larger than their prey (Brose et al., 2006). No freshwater studies have measured changes in interaction strength when the consumers are disproportionately larger than their resource, i.e. macro-invertebrates and microalgae. Thus, mesocosm were used to test whether interaction strength changed with body size across morphologically different grazers, and with ontogeny within the same species. A) Smaller individuals were expected to exert weaker impacts than larger individuals; B) Caddisfly larvae were expected to exert stronger effects than Snails, which were expected to be stronger than mayfly larvae.

4 – How does interaction strength change when species are combined? (Chapter 5)

It is unclear how measures of interaction strength vary when competing species are in close proximity, and if interspecific interactions have a greater impact on interaction strength than intraspecific interactions. Using mesocosms, interaction strength was measured when morphologically similar grazer species are combined with conspecifics and heterospecifics. The experiment tested whether overall effects on the biofilm were simply additive, or if facilitation or interference occurred between

species of grazers. Competing snail species were expected to exert stronger interspecific competition than intraspecific competition.

It was predicted, according to previous studies and theory derived from modelling, that the grazer guild would display many weak interactions and only a few strong interactions with algae. Interaction strength was expected to be context dependent i.e. vary within grazer species across resource patches due to their specific feeding adaptations such as mouth part morphology. Interaction strength was expected to increase with body size both within and across species, and to decrease when similar grazer species were combined due to interference.

1.4 References

Abrams, P. A. (2001) Describing and quantifying interspecific interactions: a commentary on recent approaches. **Oikos**, 94: 209-218.

Agrawal, A. A. (1998) Algal defense, grazers, and their interactions in aquatic trophic cascades. **Acta Oecologica**, 19(4): 331-337.

Allan, J. D. (1995) **Stream ecology: Structure and function of running waters.** London: Chapman and Hall.

Alvarez, M. and Peckarsky, B. L. (2005) How do grazers affect periphyton heterogeneity in streams? **Oecologia**, 142(4): 576-587.

Alverson, A. J. and Courtney, G. W. (2002) Temporal patterns of diatom ingestion by larval net-winged midges (Diptera: Blephariceridae: *Blepharicera*). **Freshwater Biology**, 47: 2087-2097.

Armitage, P. D., Szoszkiewicz, K., Blackburn, J. H., et al. (2003) Ditch communities: a major contributor to floodplain biodiversity. **Aquatic Conservation: Marine and Freshwater Ecosystems**, 13(165): 185.

Baekken, T. (1981) Growth patterns and food habits of Baetis rhodani, Capnia pygmaea and Diura nanseni in a west Norwegian river. **Holarctic Ecology**, 4: 139-144.

Barbee, N. C. (2005) Grazing insects reduce algal biomass in a neotropical stream. **Hydrobiologia**, 532: 153-165.

Becker, G. (1990) Comparison of the Dietary-Composition of Epilithic Trichopteran Species in a 1st Order Stream. **Archiv Fur Hydrobiologie**, 120(1): 13-40.

Begon, M., Harper, J. L. and Townsend, C.R. (1996) **Ecology: individuals, populations and communities**. 3rd edition. London, Wiley Blackwell. 1068 pp.

Berlow, E. L. (1999) Strong effects of weak interactions in ecological communities. **Nature**, 398(6725): 330-334.

Berlow, E. L., Navarrete, S. A., Briggs, C. J., et al. (1999) Quantifying variation in the strengths of species interactions. **Ecology**, 80(7): 2206-2224.

Berlow, E. L., Neutel, A. M., Cohen, J. E., et al. (2004) Interaction strengths in food webs: issues and opportunities. **Journal of Animal Ecology**, 73(3): 585-598.

Biggs, B. J. F. (1996) "Patterns in benthic algae of streams". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 31-56.

Biggs, B. J. F. and Smith, R. A. (2002) Taxonomic richness of stream benthic algae: Effects of flood disturbance and nutrients. **Limnology and Oceanography**, 47(4): 1175-1186.

Borchardt, M. A. (1996) “Nutrients”. In Stevenson, R. j., Bothwell, M. L. and Lowe, R.L. (ed.) **Algal ecology: Freshwater benthic ecosytems.** London, Academic Press. **Chapter 7:** 184-228.

Bott, T. L. (1983) "Primary productivity in streams". In Barnes, J. R. and Minshall, G. W. (ed.) **Stream Ecology.** New York, Plenum. pp. 29-53.

Bowes, M. J., Leach, D. V. and House, W. A. (2005) Seasonal nutrient dynamics in a chalk stream: the River Frome, Dorset, UK. **Science of the Total Environment**, 336(1-3): 225-241.

Bronmark, C., Rundle, S. D. and Erlandsson, A. (1991) Interactions Between Fresh-Water Snails And Tadpoles - Competition And Facilitation. **Oecologia**, 87(1): 8-18.

Brose, U., Jonsson, T., Berlow, E. L., et al. (2006) Consumer-resource body-size relationships in natural food webs. **Ecology**, 87(10): 2411-2417.

Brown, G. G., Norris, R. H., Maher, W. A., et al. (2000) Use of electricity to inhibit macroinvertebrate grazing of epilithon in experimental treatments in flowing waters. **Journal of the North American Benthological Society**, 19(1): 176-185.

Bunte, K. and Abt, S. R. (2001). Sampling surface and subsurface particle-size distributions in wadable gravel- and cobble-bed streams for analyses in sediment transport, hydraulics and streambed monitoring. Fort Collins, USA, Rocky Mountains Research Station, USDA.

Burkholder, J. M. (1996) "Interactions of benthic algae with substrate". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 253-297.

Cattaneo, A. and Mousseau, B. (1995) Empirical analysis of the removal rate of periphyton by grazers. **Oecologia**, 103: 249-254.

Cereghino, R. (2002) Shift from a herbivorous to a carnivorous diet during the larval development of some Rhyacophila species (Trichoptera). **Aquatic Insects**, 24(2): 129-135.

Chalcraft, D. R. and Resetarits, W. J. (2003) Predator identity and ecological impacts: Functional redundancy or functional diversity? **Ecology**, 84(9): 2407-2418.

Chase, J. M., Wilson, W. G. and Richards, S. A. (2001) Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. **Ecology Letters**, 4: 304-312.

Cohen, J. E., Pimm, S. L., Yodzis, P., et al. (1993) Body sizes of animal predators and animal prey in food webs. **Journal of Animal Ecology**, 62: 67-78.

Culp, J. M. and Scrimgeour, G. J. (1993) Size-Dependent Diel Foraging Periodicity of a Mayfly Grazer in Streams with and without Fish. **Oikos**, 68(2): 242-250.

Cummins, K. W. (1973) Trophic relations of aquatic insects. **Annual review of entomology**, 18: 183-206.

Cummins, K. W. and Klug, M. J. (1979) Feeding ecology of stream invertebrates. **Annual Review of Ecology, Evolution, and Systematics**, 10: 147-172.

Darwin, C. (1859) **The origin of species.** New York: The Modern Library.

De Ruiter, P. C., Neutel, A. M. and Moore, J. C. (1995) Energetics, patterns of interaction strength, and stability in real ecosystems. **Science**, 269: 1257-1260.

DeNicola, D. M. and McIntire, C. D. (1991) Effects of Hydraulic Refuge and Irradiance on Grazer-Periphyton Interactions in Laboratory Streams. **Journal of the North American Benthological Society**, 10(3): 251-262.

Diaz Villanueva, V. and Albarino, R. (2003) Algal ingestion and digestion by two Ephemopteran larvae from a Patagonian Andean stream. **Research update on Ephemoptera and Plecoptera**: 469-475.

Dodds, W. K. and Biggs, B. J. F. (2002) Water velocity attenuation by stream periphyton and macrophytes in relation to growth form and architecture. **Journal of the North American Benthological Society**, 21(1): 2-15.

Edwards, F. K. (2005). Fish presence and the ecology of stream invertebrate predators. PhD thesis, University of Edinburgh**:** 243.

Elton, C. (1927) **Animal ecology.** London: Sidgwick & Jackson Ltd.

Emmerson, M., Bezemer, T. M., Hunter, M. D., et al. (2004) How does global change affect the strength of trophic interactions? **Basic and Applied Ecology**, 5(6): 505-514.

Feminella, J. W. and Hawkins, C. P. (1995) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. **Journal of the North American Benthological Society**, 14(4): 465-509.

Flecker, A. S. (1992) Fish trophic guilds and the structure of a tropical stream: weak direct versus strong indirect effects. **Ecology**, 73: 927-940.

Gresens, S. E. (1995) Grazer Diversity, Competition And The Response Of The Periphyton Community. **Oikos**, 73(3): 336-346.

Hellawell, J. M., Leatham, H. and Williams, G. I. (1974) The upstream migratory behaviour of salmonids in the River Frome, Dorset. **Journal of Fish Biology**, 6: 729-744.

Hill, W. (1996) "Effects of light". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 184-218.

Hill, W. R. and Knight, A. W. (1987) Experimental analysis of the grazing interaction between a mayfly and stream algae. **Ecology**, 68(6): 1955-1965.

Hill, W. R. and Knight, A. W. (1988) Concurrent Grazing Effects of 2 Stream Insects on Periphyton. **Limnology and Oceanography**, 33(1): 15-26.

Hillebrand, H. (2008) Grazing regulates the spatial variability of periphyton biomass. **Ecology**, 89(1): 165-173.

Holomuzki, J. R. and Biggs, B. J. F. (2006) Food limitation affects algivory and grazer performance for New Zealand stream macro-invertebrates. **Hydrobiologia**, 561: 83-94.

Holt, R. D. (1977) Predation, apparent competition and the structure of prey communities. **Theoretical Population Biology**, 12: 495-520.

Ings, T.C., Montoya, J.M., Bascompte, J., et al. (2009) Ecological networks – beyond food webs. **Journal of animal ecology,** 78: 253-269.

Johnson, R. E., Tuchman, N. C. and Peterson, C. G. (1997) Changes in the vertical microdistribution of diatoms within a developing periphyton mat. **Journal of the North American Benthological Society**, 16(3): 503-519.

Jordan, J. and Lake, P. S. (1996) Grazer-epilithon interactions in an Australian upland stream. **Marine And Freshwater Research**, 47(6): 831-835.

Karlsson, P., Jonsson, T. and Jonsson, A. (2007) Food web structure and interaction strength pave the way for vulnerability to extinction. **Journal of Theoretical Biology**, 249: 77-92.

King-Lotufo, E. C., Brown, K. M. and Carman, K. R. (2002) The influence of periphyton biomass and density on grazing in Physella virgata. **Hydrobiologia**, 482(1-3): 23-29.

Kohler, S. L. (1992) Competition and the structure of a benthic stream community. **Ecological Monographs**, 62(2): 165-188.

Ladle, M. and Bass, J. A. B. (1981) The ecology of a small chalk stream and its response to drying during drought conditions. **Archiv fur Hydrobiologie**, 90: 448-466.

Lamberti, G. A. (1996) "The role of periphyton in benthic food webs". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 533-573.

Lamberti, G. A., Feminella, J. W. and Resh, V. H. (1987) Herbivory and intraspecific competition in a stream caddisfly population. **Oecologia**, 73(1): 75-81.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., et al. (1989) Productive Capacity of Periphyton as a Determinant of Plant Herbivore Interactions in Streams. **Ecology**, 70(6): 1840-1856.

Lamberti, G. A. and Resh, V. H. (1983) Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. **Ecology**, 64(5): 1124-1135.

Lancaster, J., Bradley, D. C., Hogan, A., et al. (2005) Intraguild omnivory in predatory stream insects. **Journal of Animal Ecology**, 74(4): 619-629.

Laska, M. S. and Wootton, J. T. (1998) Theoretical concepts and empirical approaches to measuring interaction strength. **Ecology**, 79(2): 461-476.

Ledger, M. E., Harris, R. M. L., Armitage, P. D., et al. (2008) Disturbance, patchiness and coexistence in freshwater algal communities. **Oecologia**, 155: 809-819.

Ledger, M. E., Harris, R. M. L., Milner, A. M., et al. (2006) Disturbance, biological legacies and community development in stream mesocosms. **Oecologia**, 148: 682-691.

Lodge, D.M., Cronin, G., van Donk, E., et al. (1998) “Impact of herbivory on plant standing crop: comparisons among biomes, between vascular and nonvascular plants, and among freshwater herbivore taxa”. In Jeppesen, E., Soendergaard, M. and Soendergaard M. (eds) **Structuring role of submerged macrophytes in lakes.** New York, Springer. **Chapter 8:** 149-174.

Liess, A. and Hillebrand, H. (2004) Invited review: Direct and indirect effects in herbivore periphyton interactions. **Archiv Fur Hydrobiologie**, 159(4): 433-453.

Liess, A. and Kahlert, M. (2007) Gastropod grazers and nutrients, but not light, interact in determining periphytic algal diversity. **Oecologia**, 152(1): 101-111.

Lima, S. L. and Dill, L. M. (1990) Behavioural decisions made under the risk of predation: a review and prospectus. **Canadian Journal of Zoology**, 68: 619-640.

Lindemann, R. L. (1942) The trophic-dynamic aspect of ecology. **Ecology**, 23: 399-418.

Malmqvist, B., Sjostrom, P. and Frick, K. (1991) The diet of two species of Isoperla (Plecoptera, Perlodidae) in relation to season, site and sympatry. **Hydrobiologia**, 213(3): 191-203.

Marker, A. F. H. (1976a) The benthic algae of some streams in southern England: I. Biomass of the epilithon in some small streams. **Journal of ecology**, 64(1): 343-358.

Marker, A. F. H. (1976b) The benthic algae of some streams in southern England: II. The primary production of the epilithon in a small chalk-stream. **Journal of ecology**, 64(1): 359-373.

McCann, K. (2000) The diversity-stability debate. **Nature**, 228-233.

McCann, K., Hastings, A. and Huxel, G. R. (1998) Weak trophic interactions and the balance of nature. **Nature**, 395: 794-798.

McCormick, P. V. (1994) Evaluating the Multiple Mechanisms Underlying Herbivore Algal Interactions in Streams. **Hydrobiologia**, 291(1): 47-59.

McCormick, P. V. and Stevenson, R. J. (1991) Mechanisms of Benthic Algal Succession in Lotic Environments. **Ecology**, 72(5): 1835-1848.

McIntosh, A. R. and Townsend, C. R. (1996) Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a tropic cascade mediated by fish-induced changes to grazer behaviour? **Oecologia**, 108: 174-181.

McNeely, C., Finlay, J. C. and Power, M. E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. **Ecology**, 88(2): 391-401.

Menge, B. A., Berlow, E. L., Blanchette, C. A., et al. (1994) The Keystone Species Concept - Variation in Interaction Strength in a Rocky Intertidal Habitat. **Ecological Monographs**, 64(3): 249-286.

Menge, B. A., Daley, B. A., Lubchenco, J., et al. (1999) Top-down and bottom-up regulation of New Zealand rocky intertidal communities. **Ecological Monographs**, 69(3): 297-330.

Minshall, G. W. (1978) Autrotrophy in stream ecosystems. **Bioscience**, 28: 767-771.

Moore, J. W. (1975) The role of algae in the diet of Asellus aquaticus L. and Gammarus pulex L. **Journal of Animal Ecology**, 44: 719- 730.

Neutel, A. M., Heesterbeek, J. A. P. and De Ruiter, P. C. (2002) Stability in real food webs: weak links in long loops. **Science**, 296: 1120-1123.

Osenberg, C. W., Sarnelle, O., Cooper, S. D., et al. (1999) Resolving ecological questions through meta-analysis: goals, metrics, and models. **Ecology**, 80(4): 1105-1117.

Paine, R. T. (1992) Food-Web Analysis through Field Measurement of Per-Capita Interaction Strength. **Nature**, 355(6355): 73-75.

Peckarsky, B. L. and McIntosh, A. R. (1998) Fitness and community consequences of avoiding multiple predators. **Oecologia**, 113: 565-576.

Peterson, C. G. (1996) "Response of algae to natural disturbance". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 375-402.

Peterson, C. G. and Jones, T. L. (2003) Diatom viability in insect fecal material: comparison between two species, Achnanthidium lanceolatum and Synedra ulna. **Hydrobiologia**, 501(1-3): 93-99.

Peterson, C. G., Vormittag, K. A. and Valett, H. M. (1998) Ingestion and digestion of epilithic algae by larval insects in a heavily grazed montane stream. **Freshwater Biology**, 40(4): 607-623.

Poff, N. L., Wellnitz, T. and Monroe, J. B. (2003) Redundancy among three herbivorous insects across an experimental current velocity gradient. **Oecologia**, 134(2): 262-269.

Quince, C., Higgs, P. G. and McKane, A. J. (2005) Topological structure and interaction strengths in model food webs. **Ecological modelling**, 187: 389-412.

Rosemond, A. D., Mulholland, P. J. and Elwood, J. W. (1993) Top-Down and Bottom-up Control of Stream Periphyton - Effects of Nutrients and Herbivores. **Ecology**, 74(4): 1264-1280.

Rosenfeld, J. S. (1997) The effect of large macroinvertebrate herbivores on sessile epibenthos in a mountain stream. **Hydrobiologia**, 344: 75-79.

Rosi-Marshall, E. J. and Wallace, J. B. (2002) Invertebrate food webs along a stream resource gradient. **Freshwater Biology**, 47(1): 129-141.

Sala, E. and Graham, M. H. (2002) Community-wide distribution of predator-prey interaction strength in kelp forests. **Proceedings of the National Academy of Sciences of the United States of America**, 99(6): 3678-3683.

Sear, D. A., Armitage, P. D. and Dawson, F. H. (1999) Groundwater dominated rivers. **Hydrological Processes**, 13(3): 255-276.

Siegfried, C. A. and Knight, A. W. (1976) Trophic relations of Acroneuria (Calineuria) californica (Plecoptera: Perlidae) in a Sierra foothill stream. **Environmental Entomologist**, 5: 575-581.

Steinman, A. D. (1996) "Effects of grazers on freshwater benthic algae". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 341-366.

Steinman, A. D., McIntire, C. D., Gregory, S. V., et al. (1987) Effects of herbivory type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. **Journal of the North American Benthological Society**, 6(3): 175-188.

Strong, D.R. (1992) Are trophic cascades all wet? Differentiation and doinor control in speciose ecosystems. **Ecology**, 73(3): 747-754.

Sumner, W. T. and McIntire, C. D. (1982) Grazer-periphyton interactions in laboratory streams. **Archiv Fur Hydrobiologie**, 93(2): 135-157.

Tall, L., Cloutier, L. and Cattaneo, A. (2006) Grazer-diatom size relationships in an epiphytic community. **Limnology And Oceanography**, 51(2): 1211-1216.

Taylor, B. W., McIntosh, A. R. and Peckarsky, B. L. (2002) Reach-scale manipulations show invertebrate grazers depress algal resources in streams. **Limnology and Oceanography**, 47(3): 893-899.

Taylor, D.J., Green, N.P.O. and Stout, D.W. (1997) **Biological science 1 & 2**. 3rd edition. Cambridge University Press. 992 pp.

Tikkanen, P., Muotka, T. and Huhta, A. (1994) Predator detection and avoidance by lotic mayfly nymphs of different size. **Oecologia**, 99(252): 259.

Tuchman, N. C. and Stevenson, R. J. (1991) Effects of Selective Grazing by Snails on Benthic Algal Succession. **Journal of the North American Benthological Society**, 10(4): 430-443.

Underwood, G. J. C. and Thomas, J. D. (1990) Grazing Interactions between Pulmonate Snails and Epiphytic Algae and Bacteria. **Freshwater Biology**, 23(3): 505-522.

Wetzel, R. G. (1983) **Periphyton of freshwater ecosystems.** The Hague: Dr. W. Junk Publishers.

Whittaker, R. H. (1975) **Communities an ecosystems.** New York: Macmillan.

Wootton, J. T. (1997) Estimates and tests of per capita interaction strength: diet, abundance, and impact of intertidally foraging birds. **Ecological Monographs**, 67(1): 45-64.

Wootton, J. T. and Emmerson, M. C. (2005) Measurement of interaction strength in nature. **Annual Review of Ecology, Evolution, and Systematics**, 36: 419-444.

Wotton, R. S. and Malmqvist, B. (2001) Feces in aquatic ecosystems. **Bioscience**, 51(7): 537-544.

Yodzis, P. (1981) The stablility of real ecosystems. **Nature**, 289: 674-686.

Yoshida, T., Toda, T., Kuwahara, V., et al (2004) Rapid response to changing light environments of the calanoid copepod *Calanus sinicus*. **Marine Biology**, 145(3): 505-513.

CHAPTER 2 - DISTRIBUTION OF INTERACTION STRENGTHS AMONG MACRO-INVERTEBRATE GRAZERS

2.1 Summary

Ecological theory suggests that species interactions, i.e. the impact of one species on the abundance of a prey species, are predictably distributed within food webs, where higher frequencies of weak interactions are expected compared to strong impacts. Macro-invertebrates in a lowland chalk stream were experimentally manipulated to determine the distribution of interaction strengths across an herbivore guild. Herbivores were classified into dominantly grazing or non-grazing. Weak connections dominated algal-herbivore interactions, and grazing herbivores exerted more frequent negative impacts on biofilms than non-grazing herbivores. Particularly for the snail *Theodoxus fluviatilis* which, exerted a very strong effect on algal biomass and cell abundance.

2.2 Introduction

2.2.1 *Connectance, web links and interaction strength*

Ecological literature suggests that quantifying the magnitude of the effect of one species on another is an important prerequisite for interpreting the relationship between food web structure and dynamics (Paine, 1992; Wootton, 1997; Laska and Wootton, 1998; Berlow et al., 1999, 2004). However, deriving interaction strengths within real food webs from empirical manipulations is notoriously difficult (Abrams, 2001), as it usually involves complete deletion or removal of a predatory species in order to assess its impact on the target species, such as in Paine (1992), Robson (1996), Sala and Graham (2002) and Lepak et al. (2006). Removal experiments may be affected by the duration of the experiment, for example over sufficient time indirect pathways may establish that dampen the effects being measured. Additionally, the diversity and density of the focal species may also contribute to increased variability of estimates. Thus, species manipulations of this kind are relatively idealistic, and caution must be employed when extrapolating this data to natural communities where prey (organisms being consumed) and predators (organisms consuming prey organisms) are numerous (Abrams, 2001). Consequently, inferences regarding food web dynamics derived from empirical estimates of interaction strength are perhaps more useful as a tool for determining the distribution of interactions within ecological networks (Wootton, 1997, McCann et al., 1998). Distributions of species effects are generally described as having a log normal or skewed distribution (Wootton, 1997; Sala and Graham, 2002). Theoretical and empirical evidence explicitly conclude that most species interactions will be weak

(Berlow et al., 1999; Sala and Graham, 2002; Berlow et al., 2004; Wootton and Emmerson, 2005). Weak interactions may reflect resource partitioning and trophic omnivory, which are prevalent in many food webs (Diehl, 2003; Thomson et al., 2007). When a consumer divides its feeding across several prey species, its impact on any one resource is often relatively weak (May, 1973; Paine, 1980; Begon et al., 1996). High frequencies of weak interactions contribute to ecological stability by reducing the chance of chaotic dynamics between prey and predators, thus dampening effects of stronger interactions (Wootton, 1997; McCann et al., 1998; Kokkoris et al., 2002). Furthermore, link strengths within a suite of predators may vary depending on the ecological context of the interaction; examples of context dependency include, ontogenetic shifts in either the prey, predator or a third species (Berlow et al., 2004), habitat heterogeneity and the spatial distribution of resources (Robson and Barmuta, 1998), or frequency and intensity of abiotic disturbance (Downes et al., 1998).

2.2.2 *Estimation of interaction strength*

Empirical measurement and estimation of interaction strength tends to differ from what theoreticians describe as an effect magnitude (Laska and Wootton, 1998). Theoretically derived metrics quantify single links between individuals, while empirical approaches usually quantify community level effects (Berlow et al., 1999, 2004). The ability to physically measure individual links is both logistically difficult and unlikely to be detectable. Therefore, empirical approaches that attempt to unite with theory are more desirable. Moreover, there is a requirement to be explicit about which metrics are used (Berlow et al., 2004), with the application of clear biological models or conceptual frameworks, particularly if studies that transcend ecosystems

are to be incorporated into quantitative syntheses, such as meta-analyses (Osenberg et al., 1997; Goldberg et al., 1999). Such syntheses offer a robust tool for detecting ecological signals across data sets and allow the comparison of such data.

Empirical metrics either calculate absolute (or raw difference) effects or a relative difference, either weighted by per unit biomass or per capita effects of the predatory or target species (Goldberg et al., 1999). Relative difference effects are particularly useful for empirical studies because they remove experimental confounding caused by direct effects of the environment, thus the measured response is intrinsic to that particular experimental environment (Goldberg et al., 1999). The log response ratio or 'Dynamic Index' (Laska and Wootton, 1998; Berlow et al., 1999) is a relative metric used for quantifying the impact of one species on another using the log ratio of effect, i.e. the rate of change in a target species in the presence and absence of its predator. This index behaves symmetrically about zero, and was considered by Laska and Wootton (1998) to be the only metric that estimates the theoretical coefficient of interaction. It does not assume the manipulated community is at equilibrium and is therefore ideal for assessing impacts over short time scales (Berlow et al,. 1999), although it is essential to specify time scales when using this index because the closer to the time of the perturbation the greater the response (Berlow et al., 1999 and Osenberg et al., 1999). However, studies of a short duration are also advantageous in that they minimise adverse effects of enclosures on animal behaviour (Brown et al., 1994) by reducing cage effects associated with mesocosm edges (Poff et al., 2003). Furthermore, targeted experiments of short length focus on the immediate direct response of the prey to a consumer, and this reduces the impact of indirect pathways dampening the detectable response. This flexible approach was supported by Abrams

(2001) as an alternative to longer term pulse experiments in constructing mathematical models of interaction strength.

2.2.3 *Benthic biofilms and quantification of herbivorous interactions*

A myriad of interactions can occur between benthic producers and their primary consumers within stream communities. Studies of interactions between benthic algal assemblages (prey organisms) and primary consumers (the predators of benthic algae) generally show a degree of variability, which depend on both biotic and abiotic factors, such as consumer identity (Lamberti et al., 1987; Steinman et al., 1987) and size (Rosenfeld, 1997; Poff et al., 2003), nutrient supply (McCormick, 1994; Peterson et al., 2001; Hildebrand, 2003), light availability (Johnson et al., 1997) and flow regimes (Mosisch and Bunn, 1997). Consequently, within this complex network of interactions, empirically derived interaction strength estimates are important for assessing the effect magnitude of one species (herbivores) on another (algae) in benthic communities. Periphytic biofilms are highly exploitable by primary consumers, and many invertebrates feed on the readily renewable algal assemblage and can consume high quantities of biomass (Bronmark, 1989, Rosemond et al., 1993 and Liess and Hildebrand, 2004). Therefore it could easily be assumed herbivores strongly depress algal abundance. However this is not always the case (Gresens and Lowe, 1994). For example, Cattaneo and Kalff, (1986) found a relatively small impact of small grazing chironomid larvae and oligochaete worms on algal biomass, but found grazers induced a shift in the composition of the algal assemblage from large to smaller species. Consequently, algal resource depression may depend on a) algal biofilm structure and species composition, and b) herbivore identity. In addition,

abiotic factors such as nutrient supply and irradiance also affect algal resource depression (Rosemond et al., 1993). If nutrients and light are unlimited, consumption may be offset by high algal turnover and production rates (Johnson et al., 1997). Grazing may however, enhance algal growth (Liess and Hildebrand, 2004) by restricting the growth of overstory species that limit light and nutrient availability to lower algal layers. Hence, algal community regulation depends not only on the algal community being consumed and the species consuming it, but also on the interplay between these two factors (Lamberti et al., 1987, 1989). Deciphering algal/herbivore interactions within ecological networks will give evidence of the structural significance of these interactions and perhaps the impact of bottom-up effects on the identity of keystone species.

2.2.4 *Biofilm structure and development – implications for primary consumers*

Algal assemblages are often dominated by diatoms (Tall et al., 2006), which are key components of lotic biofilms (Lamberti et al., 1989; Allan, 1995; Steinman, 1996). Chlorophyta (green algae) and Cyanophyta (blue-green algae), are frequently less abundant or diverse as diatom species but are nonetheless important members of the biofilm flora, together with other microbial organisms like fungi and bacteria (Allan, 1995). Benthic biofilms undergo successional development (McCormick and Stevenson, 1991; Allan, 1995; Steinman, 1996), with adnate algae or basal cells of filamentous forms dominating initial colonisation of substrates (Butcher, 1946). Over time these cells are succeeded by apically attached erect and stalked forms; finally filamentous species make up the overstory component (figure 2.1 modified from Steinman, 1996). Motile species, such as the naviculoid diatoms are free to move

among these different biofilm growth-forms (Johnson et al., 1997). Mature biofilms with thick layers of overstory species incur senescence of the lower levels when light and nutrients become limited for basal species (Burkholder et al., 1990; Peterson, 1996). As a result, when enough biofilm dies the upper layers can be lost as a direct result of shear stress, which sloughs the overlying algal biomass (Peterson, 1996). Following a sloughing event, biofilm development starts again (Peterson, 1996).

Implications for consumers occur depending on the particular phase of development of the biofilm. Newly established algal species are dominated by closely adhering adnate forms that show resistance to herbivores (Allan, 1995; Steinman, 1996). However, if consumed these species quickly reproduce to compensate for consumptive losses. As the biofilm develops, with the introduction of erect and stalked forms, higher consumptive losses might be expected as these are more readily consumed by herbivores (Jacoby, 1987; Tuchman and Stevenson, 1991; Allan, 1995; Steinman, 1996). Once filamentous overstory species develop herbivory may be restricted to epiphytic species as larger algae reach a developmental refugium from invertebrate mouthparts (DeNicola et al., 1990; Sarnelle et al., 1993; Steinman, 1996).

2.2.5 *Herbivore ability to crop biofilms*

The extent of herbivory varies from consumer to consumer (Poff et al., 2003). By default, biofilm consumers are to some extent generalists as they ingest non-photosynthetic heterotrophic components of the biofilm, but some species are primarily adapted to cropping benthic algae (Steinman, 1996). Lotic macroinvertebrates have been classified into functional feeding groups, using attributes such as mouthpart morphology and food acquisition (Cummins and Klug,

1979; Moog, 1995; Tachet et al., 2000). Herbivorous species originate from two functional feeding groups, 'scrapers', 'collectors' and combinations of these groups. Functional feeding groups (FFG) are based on feeding mode and behaviour. Scraper herbivores include gastropod molluscs, caddisfly larvae and mayfly larvae. Algae from biofilm layers (figure 2.1a-c) are removed by gastropods using a toothed plate (radula), and by caddisfly and mayfly larvae using sclerotised mandibles. Collector herbivores include mayfly larvae, caddisfly larvae, beetle larvae and true fly larvae (Giller and Malmqvist, 1998). Many species have brush-like mandibular appendages that browse over biofilms removing loosely attached erect / stalked algae (figure 2.1b) and some overstory species (figure 2.1c). Furthermore, body size and mode of locomotion can also influence herbivore depression of algal biomass via dislodgement (Steinman, 1996; Alvarez and Peckarsky, 2005). Consequently, large, slow moving species such as cased caddisfly larvae that drag their cases through the biofilm are likely to dislodge more algae than a light swimming mayfly larva that 'dances' across the biofilm (Steinman, 1996; Wilson et al., 1999; Poff et al., 2003).

2.2.6 *Aims of this study*

This investigation aimed to quantify the strength of interactions, using the Dynamic Index (DI), between benthic algae and a guild of herbivorous macroinvertebrates from a chalk river. The consumer taxa under study included representatives from a range of FFG's. It was hypothesised that herbivory would, 1) follow a typical interaction matrix distribution, with many weak and few strong interactions, and 2) be strongest among scrapers and weakest among collectors.

2.3 Methods

2.3.1 *Study site*

Chalk systems may be considered to be atypical of other stream types. However, with approximately 35 rivers in the South and East of England (UK biodiversity action plan www.ukbap.org.uk/UKPlans.aspx?ID=25) they are not only important ecologically but also economically, for example through abstraction and recreational fishing permits. Lowland chalk catchments, such as the River Frome in Dorset, support biologically diverse communities, which provide excellent opportunities for ecological study. Fed by a thermodynamically stable chalk aquifer, flow and discharge fluctuations are free from extremes, making this river a high-quality system to study. Significant work has been carried out on the River Frome and surrounding catchment with well over 30 years of extensive research investigating; fish (Hellawell et al., 1974), physiochemistry (Bowes et al., 2005), hydrogeology/hydrology (Arnott, 2008), invertebrates (Pardo and Armitage, 1997; Ladle et al., 1977; Ledger et al.; 2006, 2008), and algae (Marker, 1976a, 1976b; Marker and Casey, 1982; Marker et al., 1986). The Freshwater Biological Association and others have conducted river research there since 1965 (Fogg, 1979). The Mill Stream, the study site, is a 1.2 km divergent channel of the River Frome. Mean daily discharge ranges from 0.06 to 2.3 $m^3 \cdot s^{-1}$; mean suspended solids are usually low at 50 $g \cdot m^{-3}$ but can peak at 300 $g \cdot m^{-3}$; oxygen saturation ranges from 75 % to 165 %; alkalinity is about 200 $g \cdot m^{-3}$ $CaCO_3$; pH ranges from 7.8 to 8.3, nitrates from 1.0 to 3.4 $g \cdot m^{-3}$; phosphates from 0.03 to 0.16 $g \cdot m^{-3}$; water temperatures are 5.5 to 7.5 and 16 to 18.5 $^{\circ}C$ in midwinter and midsummer respectively (Hellawell et al., 1974). The section of the stream surveyed

was at East Stoke, at the Freshwater Biological Association River Laboratory (50º40'48"N, 2º11'06"W, National Grid Reference SY868868). Substrate consists of sand, fine gravel and small cobbles; macrophytes are patchily distributed and consist mainly of *Ranunculs penicillatus*, *Rorippa nasturtium aquaticum*, *Apium nodiflorum* and *Berula erecta*; banks side vegetation consists of grasses and weeds and interspersed trees, mainly *Salix* sp and *Alnus* sp (Prenda et al., 1997).

The FBA 'Fluvarium' (figure 2.2) sits over the Mill Stream and diverts the stream flow through two glass-sided channels (6.10 m long x 1.37 m wide x 0.87 m deep). It provides safe access to the natural system, whereas the main channel of the River Frome is often deep and difficult to work in. This unique facility also offers a secure building free from public vandalism, a glass roof that ensures natural light cycles, and direct access to stream water. Fluvarium based experiments are consequently 'controlled' but do not incur the restrictions associated with purely laboratory-based studies. This flowing-water aquarium opened in 1971 and provided the current author with a unique opportunity to model herbivore-biofilm interactions.

2.3.2 *Experimental biofilms and Herbivores*

Natural periphytic assemblages were allowed to colonise unglazed ceramic tiles (2.5 cm x 2.5 cm = 6.25 cm^2) for 3 weeks during June and July 2005. Tile size was selected based on the size of mesocosms (see below) and to reduce edge effects associated with artificial substrates (Brown et al., 1994). Tiles were incubated in small channels adjacent to the Mill Stream during the colonisation period. Channels were supplied with unfiltered water diverted from the Mill Stream via a network of under bank pipes. Stream flow was controlled by a series of slice valves, at the head of each

channel (see Ledger et al., 2008 for a full description). Algal propagules and stream invertebrates from the main channel were free to colonise tiles during biofilm development. Biofilms developed a characteristic green/brown layer typical of early successional periphytic mats in this system (Ledger et al., 2008). During colonisation naturally occurring herbivores were free to graze tiles to ensure experiments quantified the response of a typical algal community to herbivore removal. Herbivores were collected locally from the Mill Stream and River Frome, depending on the availability of individual taxa. Species selection was based on taxa which were known to consume benthic microalgae; either defined as a main component of their diet or in conjunction with other food sources (see Moog, 1995 and Tachet et al., 2000 for classification). These included scrapers (Gastropods and caddisfly larvae); collector-gatherers (mayfly larvae and beetle larvae); shredders, deposit and filter feeders (amphipod shrimps and mayfly larvae). Suctorial herbivores such as the cased caddis larvae, Hydroptilidae, were not included as it is difficult to quantify these effects on algal biomass. In total, 16 macro-invertebrate species or (species groups) were investigated from 14 macro-invertebrate families. These represented a large range of algal consumers, i.e. a guild of concurrently occurring herbivores in the natural system. Once collected, consumers were held in flow through arenas for at least two hours before experiments were set up, thus ensuring animal stomachs were not full prior to experimentation.

2.3.3 *Experimental design*

Grazing experiments were conducted in experimental flow-through mesocosms constructed from plastic pots (10 cm diameter x 9.5 cm depth, total surface area at base = 63.6 cm^2). Stream water passed through pots via three apertures covered by 500 μm nylon mesh. A single aperture, facing up-stream (3 cm width x 2 cm height) allowed water in, and two remaining apertures (2 cm x 2 cm) provided an exit downstream (figure 2.3). Radial holes in pot bases were coved with fine 250 μm nylon mesh. Pots were then lined with sand and tiles placed on top. This construction mimicked under-bed filtration systems, which are often employed in the aquaculture industry to reduce suspended solids and improve water quality within aquaria. This is of particular importance for mesocosm experiments where animal behaviour may be altered under stress induced by poor water quality inside enclosures. Mesocosms arranged in blocks of ten, were supported in a buoyant polystyrene frame (3 cm thick). Five conjoined replicate blocks were secured to the north fluvarium channel (figure 2.4). Environmental conditions were measured in mesocosms by taking conductivity, pH and temperature measurements 24 hours prior to experimentation (table 2.1). Two consecutive trials, each examining eight herbivore treatments and an un-grazed control treatment, were instigated during July 2005 (table 2.2). In total 16 distinct grazer treatments were tested. For each trial, replicates of one control and the eight treatments were randomly assigned to each of the five blocks, giving five control and five treatment replicates. Trials were conducted over a 70 hour period, with the exception of the *Theodoxus fluviatilis* herbivore treatment which was terminated after 23 hours. This treatment was terminated early because algal resources were rapidly depressed, and had the trial been allowed to continue, individuals of *T.*

fluviatilis may have removed the entire biofilm in considerably less time than the 70 hours. This would not have been directly comparable to other herbivore species that may have depressed resources equally to *T. fluviatilis*, but over the full 70 hours. Furthermore the effect of starvation, once resources were depleted, on both snail behaviour and the ensuing response of the biofilm was unknown and non-comparable with other treatments. An experimental period of three days (or 72 hours) was considered an appropriate time scale for a measurable effect to be detected with minimal establishment of indirect pathways leading to a dampened response. However, during experimentation it became apparent that for logistical reasons (i.e. the time required to remove experimental animals and algal scrapes) 70 hours was more appropriate and more easily managed.

Herbivores removed from the holding arenas were measured in the fluvarium using laminated graph paper and were dispensed to mesocosms in densities approximate to equal biomass across treatments (table 2.2). Estimates of treatment biomasses were established using published and unpublished (appendix 1) length mass regressions (Mason**,** 1977; Meyer, 1989; Towers, et al., 1994; Burgherr and Meyer, 1997; Benke et al., 1999). Mesocosms were checked twice daily for herbivore mortalities. Any detected mortalities were immediately replaced with fresh specimens from the holding arenas. These specimens were supplied with algal resources whilst being detained. To reduce mortalities of delicate species such as mayfly larvae, individuals were handled as little as possible using plastic pasture pipettes.

After 0.96 days (23 hours) and 2.9 days (70 hours) respectively, *T. fluviatilis* and all remaining herbivores were removed, fixed in 4% buffered formaldehyde solution and later preserved in 70% IMS with glycerol. Body dimensions (to the nearest 0.01 mm) and accurate species identity for some families of mayflies and snails were obtained

in the laboratory using a calibrated eyepiece graticule and identification keys (Holland, 1972; Macan, 1974; Hynes, 1977; Elliott et al., 1988; Wallace et al., 2003). Shell heights / widths were measured for snails, and body length or head capsule widths were measured for all other herbivores.

Tile biofilms were removed using a scalpel blade and toothbrush. The scalpel blade was used to macerate the elements of *Gongrosira incrustans* colonies as the biofilm was scraped from the tile. All residues were concentrated into 24 ml plastic scintillation vials and immediately frozen. Homogenised samples were obtained by passing the sample repeatedly through a syringe. Aliquots were removed for AFDM, Chlorophyll *a* and cell count analysis. Chlorophyll *a* ($\mu g \cdot cm^{-2}$) was estimated using 5 ml homogenised aliquots, which were freeze-dried then extracted with 90 % acetone overnight. AFDM ($\mu g \cdot cm^{-2}$) estimates were obtained from 10 ml homogenised aliquots. Samples were oven dried overnight for at least 7 hrs then ashed at 550 ^{o}C for a further 7 hrs. AFDM was calculated as the difference between sample dry weight and ashed weight, measured to the nearest 0.1 µg. A further 5 ml aliquot was used for cell count analysis to quantify grazer impacts on individual alga taxa. Algal cells were identified to the lowest taxonomic level, using appropriate identification keys and guides (Belcher and Swale, 1976; Krammer and Lange-Bertalot, 1991; Kelly, 2000). Counts were made from 5 or 25 cells depending on density (usually in the range of 100 to over 300) using an Improved Neubauer Haemocytometer. All counts were extrapolated to acquire the number of cells per cm^2.

2.3.4 *Field analysis of natural populations of the experimental taxa in the Mill Stream*

A 100 m reach of the Mill Stream was delimited and surveyed on two occasions, in July and September 2005. Ten samples were taken at random within this reach on each occasion, though only 9 of the September samples were analysed. A 0.06 m^2 Surber sampler was used, disturbing the benthos for a standard time of 60 seconds. Samples were preserved in 4% buffered formaldehyde. Samples were sorted in the laboratory, and invertebrates preserved in a 70% IMS solution with glycerol. Invertebrates were identified to the lowest taxonomic level using standard identification keys. The target species used in the experimental process previously described were enumerated and population densities calculated.

2.3.5 *Data analysis*

To standardise for potential intrinsic differences between trial biofilms, per capita interaction strengths were calculated using the Dynamic Index (DI) (Wootton, 1997 and Berlow et al., 1999). This index quantifies an effect as a log ratio of herbivore impact versus control per individual, per unit time: $DI = Ln(N/D)/Yt$

Where, N = normal (treatment), D = deleted (ungrazed control), Y = grazer abundance, t = time (days). This index is based on the discrete-time version of the dynamics described by Lotka-Volterra predator / prey-equations, and was intended to quantify an effect size theoretically equivalent to the coefficient of interaction strength (Berlow et al., 1999). Per capita impacts (DI) on biofilms were estimated at the algal community and population levels using AFDM / Chlorophyll *a*, and cell count data respectively. Dynamic index calculations for *T. fluviatilis* used a time factor of 0.96 days instead of the 2.9 day standard. Control values were not adjusted

in the DI calculation for *T. fluviatilis* as it would have little impact on the estimated DI, and maintaining consistent experimental endpoints across treatments was considered more relevant. Once DI values were calculated data were found to be normally distributed with homogeneous variances (Anderson-Darling and Levene tests respectively, $\alpha = 0.05$). Trials were analysed together using the parametric one-way analysis of variance (ANOVA) to test for significant differences in per capita impacts on algal AFDM and Chlorophyll *a* across herbivore treatments ($\alpha = 0.05$). Having detected a significant difference across groups, comparisons were made using Tukey's pairwise multiple comparisons ($\alpha = 0.05$). AFDM and Chlorophyll *a* within a treatment were analysed using one-way ANOVA to determine if herbivores depressed biomass measures differently ($\alpha = 0.05$). Individual herbivore treatments were then tested to determine if dynamic index values were significantly different from zero (i.e. no interaction) using the student t-test ($\alpha = 0.05$).

Herbivore population interaction strengths were calculated by multiplying the per capita interaction strengths by the population density (table 2.6) of that particular species within the Mill Stream. Data were found to be normally distributed with homogeneous variances (Anderson-Darling and Levene tests respectively, $\alpha = 0.05$). A one-way analysis of variance (ANOVA) was used to test for significant differences in population impacts on algal AFDM and Chlorophyll *a* across herbivore treatments ($\alpha = 0.05$). Tukey's pairwise multiple comparisons ($\alpha = 0.05$) were used to compare treatments.

Per capita effects were calculated using the dynamic index for individual algal taxa, including, Chlorophyta (mostly *Gongrosira incrustans* and Chlorococcales), Cyanophyta (predominantly *Pleurocapsa* sp. and *Oscillatoria* sp.) and Bacillariophyta (diatoms). The frequency distribution of herbivore impacts (DI) on algal cell densities

were pooled and then plotted for different classifications of herbivores, (a) all herbivores (b) predominantly 'grazing' herbivores (scrapers and collector gathers) and (c) predominantly 'non-grazing' herbivores (shredders, deposit and filter feeders). Grazers and non-grazers were classified using functional feeding guilds from Moog (1995) and Tachet et al (2000) (table 2.3). Detailed treatment effects on algal taxa were than assessed by plotting the gradient of per capita interactions across herbivore treatments for broad algal groups, and five common diatoms (*Melosira varians*, *Achnanthidium minutissimum*, *Cocconeis* spp., *Amphora* spp. and *Nitzschia* spp.)

2.4 Results

2.4.1 *Per capita and population-level herbivore impacts on algal biomass estimates*

The one-way ANOVA confirmed there was a significant difference in per capita effects across herbivore treatments on AFDM ($P = < 0.001$) and Chlorophyll *a* ($P = < 0.001$) (table 2.4). Tukey's pairwise comparisons showed that *T. fluviatilis* exerted a significantly stronger negative interaction on algal biomass estimates (AFDM and Chlorophyll *a*) compared to all other herbivores (figure 2.5). Four herbivores exerted different impacts on different algal biomass estimates. Three snails (*T. fluviatilis*, *Radix. balthica* and *Planorbis* sp.) and one mayfly (*Caenis luctuosa* grp.) significantly depressed Chlorophyll *a* compared to AFDM (figure 2.5). T-tests showed that nine of the 16 herbivores tested, significantly reduced DI below zero (table 2.5). *Theodoxus fluviatilis* and *Valvata piscinalis* had strong per capita impacts on AFDM ($P = 0.002$ for both) and Chlorophyll *a* ($P = 0.017$ and 0.004, respectively). Seven other herbivores induced significant per capita reductions on Chlorophyll *a* only (figure 2.5) i.e. *R. balthica* ($P = 0.013$), *Lepidostoma hirtum* ($P = 0.035$), *Physa fontinalis* ($P = 0.050$), *Planorbis* sp. ($P = 0.006$), *C. luctuosa* grp. ($P = 0.015$), *Serratella ignita* ($P = 0.044$) and *Baetis buceratus* ($P = 0.047$). Overall, statistically significant negative per capita impacts on algal AFDM and Chlorophyll *a* were dominated by three gastropod molluscs (*T. fluviatilis*, *R. balthica* and *P. fontinalis*) and one cased caddisfly larvae (*L. hirtum*). These species showed a difference between the strength of impact on Chlorophyll *a* and AFDM depression, with a stronger impact on the former.

The realised population-level interaction strength was dominated by the effect of the *T. fluviatilis* population on chlorophyll *a* (figure 2.6). *Potamopyrgus antipodarum* and

B. tentaculata did show a greater effect at the population level compared to their per capita effect. However, this did not statistically differ, in terms of algal depression, from the other taxa.

2.4.2. *Frequency Distributions and interaction strength gradients*

Frequency distributions of herbivore impacts across algal taxa produced a high occurrence of no effect, or weak positive and negative effects (DI category 3 in figure 2.7a) for all herbivores combined. Positive and negative effects either side of category 3 were equally distributed. Grazing herbivores also had a high frequency of weak effects (category 3). However, either side of this category, impacts were more frequently negative (figure 2.7b). Species classified as non-grazing herbivores produced a higher occurrence of positive impacts on algal abundance. But more surprisingly, strong negative impacts on algal taxa were also observed in the non-grazing herbivore category (figure 2.7.c).

Gradients of herbivore impacts varied for different algae (figure 2.8a-d), with less abundant taxa such as the Cyanophyta sustaining stronger reductions in abundance (DI up to -0.8, indicating a strong decrease in cell abundance). More abundant taxa such as *G. incrustans* and diatoms were also strongly affected, with positive and negative impacts ranging from -0.21 to +0.10 and –0.61 to +0.15 respectively. *Lepidostoma hirtum* and *C. luctuosa* group treatments strongly depressed the less abundant Cyanophyta and Chlorophyta (excluding *G. incrustans*), while negative impacts on *G. incrustans* and diatoms were dominated by gastropod molluscs. *Theodoxus fluviatilis* and *P. fontinalis* depressed both *G. incrustans* and diatoms,

while *V. piscinalis* only depressed *G. incrustans*, and *R. balthica* only depressed diatoms (figure 2.8a-d).

Diatom taxa were also affected differently by different herbivore species (figure 2.9a-e). *Melosira varians*, a chain forming overstory species, was negatively affected by gastropod molluscs (*R. balthica* and *P. fontinalis*) and positively by two non-grazing species, *Leuctra geniculata* and *L. hirtum* (figure 2.9.a). DI values for *M. varians* ranged from –0.23 to + 0.14. *Achnanthidium minutissimum*, a prostrate monoraphid diatom that attaches to the substratum via a short mucilage stalk and often forms chains in the understory, was also negatively affected by gastropods (*T. fluviatilis* and *P. fontinalis*) and positively affected by non-grazing herbivores (*Bithynia tentaculata*, *L. geniculata*, *Gammarus pulex* and *L. hirtum*). DI values ranged from –0.18 to + 0.22 (figure 2.9.b). *Cocconeis* spp. are solitary and adnate monoraphid diatoms that tightly adhere to substrates via a mucilage pad. These cells incurred negative impacts from gastropods (*P. fontinalis*, *R. balthica* and *T. fluviatilis*) while also being positively affected by non-grazing herbivores (*L. geniculata*, *B. tentaculata* and *G. pulex*) (figure 2.9.c). DI values ranged from – 0.23 to +0.11.

The *Amphora* spp. group was dominated by the small species *Amphora pediculus*. These are solitary adnate cells, which are motile and move throughout the biofilm. Negative herbivore impacts were generally weaker on this species, with the exception of *T. fluviatilis*. By comparison, the herbivore with the second strongest impact was *R. balthica*, with a DI of -0.10. Thus it was weaker at reducing *Amphora* spp. than *T. fluviatilis* (-0.24). Positive impacts on *Amphora* spp. were of a similar magnitude to other diatom species, around + 0.10 DI in the *L. geniculata* stonefly treatment (figure 2.9.d). *Nitzschia* spp. (small < 50 µm) are small araphid motile species that usually live as solitary cells in mucilage tubes, but can sometimes form chain-like or stellate

colonies. These were negatively affected by four herbivores, including three snails (*P. fontinalis*, *R. balthica* and *Planorbis* sp.) and one non-grazing herbivore (*L. hirtum*). Positive herbivore impacts were negligible for this algal group, with most impacts being negative (figure 2.9.e). DI values ranged from – 0.18 to + 0.05.

2.4.3. *Summary of herbivore impacts across algal groups*

The negative impact exerted by *T. fluviatilis* on *G. incrustans* was relatively isolated with no other herbivore capable of depressing these algae to the same extent. Negative per capita impacts on diatom abundance, across herbivores appeared to be dominated by snails such as *T. fluviatilis*, *R. balthica* and *P. fontinalis*. However, impacts on diatom taxa varied for each snail. *Melosira varians*, *Cocconeis* spp. and *Nitzschia* spp. were depressed more effectively by *P. fontinalis* than *T. fluviatilis*. However, *T. fluviatilis* had a relatively strong impact on *Cocconeis* spp. and certainly induced a greater per capita decrease in *A. minutissimum* and *Amphora* spp. by comparison to *P. fontinalis*. Interestingly, *R. balthica* appeared to exhibit a wide range of effect magnitude, with some species negatively affected and others positively or not at all; for example it had strong negative effects on most diatom taxa examined but positive effects on *G. incrustans*. Cyanophyta and *A. minutissimum* were also depressed relatively weakly by *R. balthica*. Generally, positive impacts were typically associated with the non-grazing herbivores. Species that exerted relatively little or no impact on abundances included the mayflies, and other snail species, such as the Planorbidae and *V. piscinalis*.

2.5. Discussion

2.5.1 *Distributions of per capita interactions within a guild of herbivores*

Interaction strengths across this herbivore guild were not distributed evenly, with relatively few species exerting strong impacts on algal biomass and assemblage structure. Of the few strong impacts sustained, these were dominated by grazing snails, and in particular *T. fluviatilis*. Paine (1992), and Sala and Graham (2002) also found a distribution of interaction strength skewed towards weak effects, when they investigated the interactions between a suite of herbivorous organisms and their algal prey. Paine (1992) suggests a net negative effect is typical for a group of consumers, while positive effects can result from indirect enhancement, for example, he found grazers mechanically increased substratum availability for spore attachment as they moved over the substrate. This investigation agrees with Paine's (1992) observations, because the net effect was negative despite only a few strong interactions, thus implying herbivores were consuming algal prey at a greater rate than the algal turnover. However, herbivore interactions at the algal taxon level varied more, with several algae receiving positive impacts, especially from predominantly non-grazing species such as *B. tentaculata* and *L. geniculata*. *Bithynia tentaculata* primarily consumes microphytes through filtering-feeding which, is supplemented by scraping benthic algae (Moog, 1995; Tachet et al., 2000). Positive impacts of this snail probably occurred by direct enhancement of algal resources through a combination of intermediate grazing (thus reducing the chance of biofilm senescence through light limitation), and nutrient recycling via faecal pellets (Bronmark, 1989). *Leuctra geniculata*, like *B. tentaculata* does not primarily consume algae but supplements its

shredding detritivorous diet by grazing microphytes (Moog, 1995; Tachet et al., 2000). It probably enhances algal abundance by similar mechanisms to *B. tentaculata*. Negative impacts on all but the rarest algal taxa (Chlorophyta – excluding *G. incrustans* and Cyanophyta) were dominated by grazing herbivores, particularly snails (*R. balthica*, *P. fontinalis* and *T. fluviatilis*). Snails are well equipped with a toothed radula for removing layers of an algal biofilm and consistently exert negative impacts on stalked and erect algal forms, such as *A. minutissimum* and *M. varians* (Bronmark, 1989; Steinman, 1996). Strong snail impacts are less consistently reported for adnate diatom species that adhere to the substrate (Bronmark, 1989; Lamberti et al., 1987; Steinman et al., 1989). In light of this evidence, it is surprising but not unrealistic to find negative snail impacts on the adnate diatoms, *Amphora* spp. and *Cocconeis* spp. A similar effect was reported by Munoz et al (2000). They found the freshwater snail *Stagnicola vulnerata* also decreased adnate *C. placentula* densities. Furthermore, strong negative impacts on these diatoms were limited to just three snails (*T. fluviatilis*, *R. balthica* and *P. fontinalis*). These particular snails may well be equipped to remove even the toughest diatoms, especially if they can restrict biofilm development to maintain the diatom assemblage in a loosely bound mucopolysaccharide matrix (Lawrence et al., 2002). In this condition adnate diatoms may offer less resistance to ingestion. Certainly, *T. fluviatilis* reduced algal biomass sufficiently to induce a paucity of algal cells and underdevelopment of the biofilm physiognomy. Moreover, Steinman (1991) suggests that adnate species like *Cocconeis* spp. are more susceptible to grazing when snails are hungry. This could imply that the strong impact of *T. fluviatilis* induced further reductions in adnate species driven by hunger. Nonetheless, the majority of herbivore impacts, particularly non-grazing species, infrequently depressed adnate diatoms which does support trends

in the literature (Steinman, 1996; Peterson et al., 1998; Wellnitz and Ward, 1998; Diaz Villanueva and Modenutti, 2004), and suggests that depression of specific algae is dependent on the herbivore species, and in particular the mouthpart morphology that the herbivore possesses (see chapter one for an overview).

The work of Sala and Graham (2002) used the result of a deleterious experiment to quantify per capita interaction strength for a suite of eight herbivorous species consuming algal spores. Empirically derived interaction strengths were then extrapolated to a further 37 herbivore species using a relationship derived from herbivore body mass. They found that the distribution of interactions were log-normal and skewed towards weaker interactions. Either through direct experimentation of a large range of consumers (this study) or by extrapolating the magnitude of effect related to some consumer attribute (e.g. body size, Sala and Graham, 2002), distributions follow similar patterns. Nevertheless, the identity of species that exert strong impacts may be important in different ecological contexts, for example resource limitation in Steinman's (1991) work integrates the effect of satiated versus hungry snails on resource depression. This kind of practical, but logistically difficult method of quantifying interaction strengths is essential if estimates of the effect of one species on another are to be determined across spatially and temporally diverse habitats. Context dependency does not transcend any quick-fire method of extrapolating empirical estimates of interaction strength. Integration of contextually derived consumer / prey interactions is essential for a more holistic approach. The following chapter investigates one aspect of context dependency in herbivore-algae interactions, namely biofilm structure.

2.5.2 *Are snails keystone interactors?*

Snails were by far the strongest interactors in this particular chalk stream. However, are they keystone interactors? Keystone species were originally described by Paine (1969), who suggested that these are organisms that regulate the abundance of another species that would otherwise dominate the system if they were not consumed by the keystone. However, since then the keystone concept has been refined (Power et al., 1996; Sole and Montoya, 2001 and Jordan, 2002). Firstly, a keystone species is likely to produce an effect that is much greater than could be predicted by abundance alone, i.e. a large effect with a small population (Power et al., 1996). Secondly, keystones have been described within ecological networks as being highly connected (Sole and Montoya, 2001 and Jordan, 2002). Consequently, it seems that in this stream, snails and in particular *Theodoxus fluviatilis*, which has large population and per capita effects compared to other herbivores; does consume algae as a keystone species. However, the level of connectedness of this snail is unavailable and therefore its role as a keystone species within the ecological network is unclear. Further analysis of which algal species are consumed by *T. fluviatilis* and how it is affected by predation may help identify its status within the network.

2.5.3 *How important are snail grazers across systems?*

Classic reviews of algal herbivore interactions, such as Feminella and Hawkins (1995) and Steinman (1996) clearly show that snail grazers, where they exist, exhibit strong impacts on algal biomass and assemblage structure. However, snail effects are less pronounced when compared to species of grazing cased caddisflies (Feminella and Hawkins, 1995 and Steinman, 1996). Weaker snail effects, compared to caddis,

detected by Feminella and Hawkins review (1995) could be an artefact of large ranges in snail density and biomass across the experiments. Therefore, snail distributions may correlate to snail impacts globally. Snail distributions were initially thought to be associated with water chemistry and in particular calcium ion concentrations (Dussart, 1976; Lodge et al., 1998; Yipp, 1990). Shell-bearing snails are physiologically reliant on calcium to ensure their growth, development and survivorship (Dussart, 1976 and Briers, 2003). However, Briers (2003) highlights the effects of calcium levels on snail distributions, according to what Boycott (1936) describes as calciphile and noncalciphile snails. Stating that; environmental calcium requirements are important for determining macroscale patterns in the geographical range sizes of snails. Consequently, the range size of noncalciphile species is larger than calciphile species, which frequently occur along calcareous geologies (Briers, 2003). According to Boycott (1936) *Theodoxus fluviatilis* is a calciphile, and thus requires > 20 mg L^{-1} calcium ion level. Consequently, *T. fluviatilis* is relatively abundant in the Mill Stream, where calcium concentrations are around 90 mg L^{-1} (Marker and Casey, 1982). Therefore, the strong affect of *T. fluviatilis* may be restricted by its distribution to calcium rich systems. *Radix balthica* on the other hand is a noncalciphile and has a wider geographical range, but lower calcium levels restricts snail growth and limits overlap in generations (Briers, 2003). Calcium concentration may therefore affect snail impacts on algal resources through its control on snail distribution, growth and fecundity.

Additionally, a calcium rich habitat, such as in the Mill Stream supports a diverse snail assemblage. One of the smaller species, *Potamopyrgus antipodarum* was present in very high densities (59550 m^{-2}). Consequently, the per capita impacts of this small snail were relatively small, yet the population effect was considerably larger. A

similar phenomenon occurs in the snail's native New Zealand (Holomuzki and Biggs, 1999). Thus, although body size is important for per capita impacts it is less effective at predicting population level impacts.

2.5.4 *Realised population interactions and scaling: the use of mesocosms to elucidate ecological pathways*

Scaling up per capita interaction strengths, to the population level of natural communities in the Mill Stream, indicates that the strong effect of individual *Theodoxus fluviatilis* may have implications within the community. However, a simple scaling technique such as this may not accurately demonstrate how *T. fluviatilis* will perform within the community itself, and must be used as guidance only (Thrush et al., 1997). Traditionally studies conducted in small or model systems were assumed to be scale independent and were naively applied to ecosystem-scale research and management (Schindler, 1998; Bergstrom and Englund, 2002; Bergstrom, 2004). However, these highly simplified ecological conditions are subject to the effects of scaling, both temporally and spatially. Although effects can be minimised by scaling models, such as the dimensional analysis used by Petersen and Englund (2005) or through comparing small-scale and large-scale studies over a range of scales, these techniques are often complex or logistically difficult. Moreover, it may be simpler to put model systems into an experimental context where they are used to address specific questions and not explicitly required to scale to the larger ecosystem level (Petersen and Englund, 2005). Although not always cost effective, ecosystem-level studies are perhaps best for elucidating large-scale spatial and temporal effects, while model studies offer a cheap and logistically feasible

alternative that can accurately determine the direction of processes (Kohler and Wiley, 1997, Drenner and Mazumder, 1999; Greathouse et al., 2006) and perhaps less reliably the magnitude of effect (Taylor et al., 2002; Greathouse et al., 2006).

The scope of an experimental study to predict large-scale outcomes may depend on the replicability and realism of the study being undertaken (Trunov et al., 1994; Kraufvelin, 1998; Petersen and Englund, 2005; Ledger et al., 2009). Replicability refers to the extent to which model replicates mimic each other. However, the replicability of experimental replicates may excel at the initiation of an experiment, but follow different trajectories as time progresses (Kraufvelin, 1998). Factors inducing divergence between replicates include; the natural variability in transplanted material and experimental design errors and execution (Kraufvelin, 1998). In this current study algal biofilms in particular were subject to variability during algal colonisation. However, the extent of divergence between biofilms was restricted over the relatively short time scale of this study (see Kraufvelin, 1998 who suggests that small differences in replicates may magnify over time). The realism of an experiment often depends on a trade-off between highly realistic ecosystem-specific models and generic models that test broad theories applicable across systems (Petersen and Englund, 2005). Ecosystem specific models test hypotheses linked to specific ecosystems, and generally incorporate a high level of spatial and temporal complexity (Petersen and Englund, 2005). Physiochemical variables are easily controlled (Petersen and Englund, 2005; Ledger et al., 2009), but ecological control is more taxing (Petersen and Englund, 2005). However, with an increase in realism replicability decreases (Kohler and Wiley, 1997). Furthermore, large scale studies are expensive and often difficult to execute (Drenner and Mazumder, 1999). Generic models on the other hand are small, cost effective, easier to replicate and control, but

lack ecosystem realism (Petersen and Englund, 2005). The experimental units in this study provided an opportunity to assess baseline estimates of interaction strength, both at a per capita level and the realised population level. With a reduction in realism it is important to evaluate these results cautiously: we may be confident in the direction of effect but more wary of the magnitude of effect. As an addition to this work it would be beneficial to identify aspects of the natural history of the animals tested, such as reproductive rates, life cycles, and behaviour at different scales (Thrush et al., 1997). This information could then be used to evaluate scale effects associated with this experimental design.

Overall, mesocosms offer ecologists simple generic tools to elucidate important ecological pathways and processes, which must not be ignored as unrealistic, but considered in the right experimental context.

2.6 References

Abrams, P. A. (2001) Describing and quantifying interspecific interactions: a commentary on recent approaches. **Oikos**, 94: 209-218.

Allan, J. D. (1995) **Stream ecology: Structure and function of running waters.** London: Chapman and Hall.

Alvarez, M. and Peckarsky, B. L. (2005) How do grazers affect periphyton heterogeneity in streams? **Oecologia**, 142(4): 576-587.

Arnott, S. (2008) **An assessment of methods for catchment-scale identification of groundwater-surface water interactions in lowland permeable catchments (PhD).** Exeter: University of Exeter.

Begon, M., Harper, J. L. and Townsend, C.R. (1996) **Ecology: individuals, populations and communities**. 3rd edition. London, Wiley Blackwell. 1068 pp.

Belcher, H. and Swale, E. (1976) **A beginner's Guide to freshwater algae.** London: Her Majesty's Stationery Office.

Benke, A. C., Huryn, A. D., Smock, L. A., et al. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. **Journal of the North American Benthological Society**, 18(3): 308-343.

Bergstrom, U. (2004). Spatial heterogeneity and biotic interactions - scaling from experiments to natural systems. Department of ecology and environmental science, University of Sweden.

Bergstrom, U. and Englund, G. (2002) estimating predation rates in experimental systems: scale-dependent effects of aggregated behaviour. **Oikos**, 97: 251-259.

Berlow, E. L., Navarrete, S. A., Briggs, C. J., et al. (1999) Quantifying variation in the strengths of species interactions. **Ecology**, 80(7): 2206-2224.

Berlow, E. L., Neutel, A. M., Cohen, J. E., et al. (2004) Interaction strengths in food webs: issues and opportunities. **Journal of Animal Ecology**, 73(3): 585-598.

Bowes, M. J., Leach, D. V. and House, W. A. (2005) Seasonal nutrient dynamics in a chalk stream: the River Frome, Dorset, UK. **Science of the Total Environment**, 336(1-3): 225-241.

Briers, R. A. (2003) Range size and environmental calcium requirements of British freshwater gastropods. **Global ecology and biogeography**, 12: 47-51.

Bronmark, C. (1989) Interactions between epiphytes, macrophytes and freshwater snails: a review. **Journal of Molluscan Studies**, 55: 299-311.

Brown, K. M., Carman, K. R. and Inchausty, V. (1994) Density-dependent influences on feeding metabolism in a freshwater snail. **Oecologia**, 99: 158-165.

Burgherr, P. and Meyer, E. I. (1997) Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. **Archiv Fur Hydrobiologie**, 139(1): 101-112.

Burkholder, J. M., Wetzel, R. G. and Klomparens, K. L. (1990) Direct comparison of phosphate uptake be adnate and loosely attached microalgae within an intact biofilm matrix. **Applied and Environmental Microbiology**, 56(9): 2882-2890.

Butcher, R. W. (1946) Studies in the ecology of Rivers: VI. The algal growth in certain highly calcareous streams. **Journal of ecology**, 33(2): 268-283.

Boycott, A. E. (1936) The habitats of the freshwater Mollusca in Britain. **Journal of Animal Ecology**, 5: 116-186.

Cattaneo, A. and Kalff, J. (1986) The effect of grazer size manipulation on periphyton communities. **Oecologia**, 69: 612-617.

Chase, J. M., Abrams, P. A., Grover, J. P., et al. (2002) The interaction between predation and competition: a review and synthesis. **Ecology Letters**, 5(2): 302-315.

Cummins, K. W. and Klug, M. J. (1979) Feeding ecology of stream invertebrates. **Annual Review of Ecology, Evolution, and Systematics**, 10: 147-172.

DeNicola, D. M., McIntire, C. D., Lamberti, G. A., et al. (1990) Temporal patterns of grazer-periphyton interactions in laboratory streams. **Freshwater Biology**, 23: 475-489.

Diaz Villanueva, V. and Modenutti, B. (2004) Experimental analysis of grazing by the mayfly Meridialaris chiloeensis on different successional stages of stream periphyton. **International Review of Hydrobiology**, 89(3): 263-277.

Diehl, S. (2003) The evolution and maintenance of omnivory: Dynamic constraints and the role of food quality. **Ecology**, 84(10): 2557-2567.

Drenner, R. W. and Mazumder, A. (1999) Microcosm experiments have limited relevance for community and ecosystem ecology: comment. **Ecology**, 80(3): 1081-1085.

Downes, B. J., Lake, P. S., Glaister, A., et al. (1998) Scales and frequencies of disturbances: rock size, bed packing and variation among upland streams. **Freshwater Biology**, 40(4): 625-639.

Dussart, G. B. J. (1976) The ecology of fresjwater mollusks in North-west England in relation to water chemistry. **Journal of molluscan studies**, 42: 181-198.

Elliott, J. M., Humpesch, U. H. and Macan, T. T. (1988) **Larvae of the British Ephemeroptera: a key with ecological notes. Scientific Publication No. 49.** Ambleside: Freshwater Biological Association.

Feminella, J. W. and Hawkins, C. P. (1995) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. **Journal of the North American Benthological Society**, 14(4): 465-509.

Fogg, G. E. (1979) **Freshwater Biological Association: the first firty years 1929 - 1979.** Ambleside: Freshwater Biological Association.

Giller, P. S. and Malmqvist, B. (1998) **The biology of streams and rivers.** Oxford: Oxford university press.

Goldberg, D. E., Rajaniemi, T., Gurevitch, J., et al. (1999) Empirical approaches to quantifying interaction intensity; competition and facilitation along productivity gradients. **Ecology**, 80(4): 1118-1131.

Greathouse, E. A., Pringle, C. M., and McDowell, W. H. (2006) Do small-scale exclosure/enclosure experiments predict the effects of large-scale extirpation of freshwater migratory fauna? **Oecologia**, 149: 709-717.

Gresens, S. E. and Lowe, R. L. (1994) Periphyton Patch Preference in Grazing Chironomid Larvae. **Journal of the North American Benthological Society**, 13(1): 89-99.

Hellawell, J. M., Leatham, H. and Williams, G. I. (1974) The upstream migratory behaviour of salmonids in the River Frome, Dorset. **Journal of Fish Biology**, 6: 729-744.

Hillebrand, H. (2003) Opposing effects of grazing and nutrients on diversity. **Oikos**, 100(3): 592-600.

Holland, D. G. (1972) **A key to the larvae, pupae and adults of the British species of Elminthidae.** Ambleside: Freshwater Biological Association.

Holomuzki, J. R. and Biggs, B. J. F. (1999) Distributional responses to flow disturbance by a stream- dwelling snail. **Oikos**, 87(1): 36-47.

Hughes, A. R. and Grabowski, J. H. (2006) Habitat context influences predator interference interactions and the strength of resource partitioning. **Oecologia**, 149(2): 256-264.

Hynes, H. B. N. (1977). A key to the adults and nymphs of the British stoneflies (Plecoptera) with notes on their ecology and distribution. Ambleside, Freshwater Biological Association.

Jacoby, J. M. (1987) Alterations in periphyton characteristics due to grazing in a cascade foothill stream. **Freshwater Biology**, 18(3): 495-508.

Johnson, R. E., Tuchman, N. C. and Peterson, C. G. (1997) Changes in the vertical microdistribution of diatoms within a developing periphyton mat. **Journal of the North American Benthological Society**, 16(3): 503-519.

Jonsson, T., Karlsson, P. and Jonsson, A. (2006) Food web structure affects the extinction risk of species in ecological communities. **Ecological Modelling**, 199(1): 93-106.

Jordan, F. (2002) Searching for keystones in ecological networks. **Oikos**, 99(3): 607-612.

Kelly, M. (2000) Identification of common benthic diatoms in rivers. **Field studies**, 9: 583-700.

Kohler, S. L. and Wiley, M. J. (1997) Pathogen outbreaks reveal large-scale effects of competition in stream communities. **Ecology**, 78(7): 2164-2176.

Kokkoris, G. D., Jansen, V. A. A., Loreau, M., et al. (2002) Variability in interaction strength and implications for biodiversity. **Journal of Animal Ecology**, 71(2): 362-371.

Krammer, K. and Lange-Bertalot, H. (1991) **Susswasserflora von mitteleuropa, Bd 2/1 - 2/5: Bacillariophyceae.** Gustav Fischer Verlang.

Kraufvelin, P. (1998) Model ecosystem replicability challenged by the 'soft' reality of a hard bottom mesocosm. **Journal of Experimental Marine Biology and Ecology**, 222: 247-267.

Ladle, M., Bass, J. A. B., Philpott, F. R., et al. (1977) Observations on ecology of Simuliidae from River Frome, Dorset. **Ecological Entomology**, 2(3): 197-204.

Lamberti, G. A., Ashkenas, L. R., Gregory, S. V., et al. (1987) Effects of three herbivores on periphyton communities in laboratory streams. **Journal of the North American Benthological Society**, 6(2): 92-104.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., et al. (1989) Productive Capacity of Periphyton as a Determinant of Plant Herbivore Interactions in Streams. **Ecology**, 70(6): 1840-1856.

Laska, M. S. and Wootton, J. T. (1998) Theoretical concepts and empirical approaches to measuring interaction strength. **Ecology**, 79(2): 461-476.

Lawrence, J. R., Scharf, B., Packroff, G., et al. (2002) Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. **Microbial Ecology**, 44(3): 199-207.

Ledger, M. E., Harris, R. M. L., Armitage, P. D., et al. (2008) Disturbance, patchiness and coexistence in freshwater algal communities. **Oecologia**, 155: 609-819.

Ledger, M. E., Harris, R. M. L., Armitage, P. D., et al. (2009) Realism of model ecosystems: an evaluation of physico-chemistry and macroinvertebrate assemblages in artificial streams. **Hydrobiologia**, 617: 91-99.

Ledger, M. E., Harris, R. M. L., Milner, A. M., et al. (2006) Disturbance, biological legacies and community development in stream mesocosms. **Oecologia**, 148: 682-691.

Lepak, J. M., Kraft, C. E. and Weldel, B. C. (2006) Rapid food web recovery in response to removal of an introduced apex predator. **Canadian Journal Of Fisheries And Aquatic Sciences**, 63(3): 569-575.

Liess, A. and Hillebrand, H. (2004) Invited review: Direct and indirect effects in herbivore periphyton interactions. **Archiv Fur Hydrobiologie**, 159(4): 433-453.

Lodge, D. M., Brown, K. M., Klosiewski, S. P., et al (1987) Distribution of freshwater snails: spatial scale and the relative importance of physiochemical and biotic factors. **American Mallacological Bulletin**

Macan, T. T. (1994) **A key to the British fresh- and brackish-water gastropods with notes on their ecology. Scientific publication No. 13.** Ambleside: Freshwater Biological Association.

Marker, A. F. H. (1976a) The benthic algae of some streams in southern England: I. Biomass of the epilithon in some small streams. **Journal of ecology**, 64(1): 343-358.

Marker, A. F. H. (1976b) The benthic algae of some streams in southern England: II. The primary production of the epilithon in a small chalk-stream. **Journal of ecology**, 64(1): 359-373.

Marker, A. F. H. and Casey, H. (1982) The population and production dynamics of benthic algae in an artificial recirculating hard-water stream. **Phil. Trans. R. Soc. Lond. B**, 298: 265-308.

Marker, A. F. H., Clarke, R. T. and Rother, J. A. (1986) Changes in an epilithic population of diatoms, grazed by chironomid larvae, in an artificial recirculating stream. **9th Diatom-symposium 1986**: 143-149.

Mason, C. F. (1977) Populations and production of benthic animals in two contrasting shallow lakes in Norfolk. **Journal of Animal Ecology**, 46: 147-172.

May, R. M. (1973) **Stability and complexity in model ecosystems.** Princeton: Princeton University Press.

McCann, K., Hastings, A. and Huxel, G. R. (1998) Weak trophic interactions and the balance of nature. **Nature**, 395: 794-798.

McCormick, P. V. (1994) Evaluating the Multiple Mechanisms Underlying Herbivore Algal Interactions in Streams. **Hydrobiologia**, 291(1): 47-59.

McCormick, P. V. and Stevenson, R. J. (1991) Mechanisms of Benthic Algal Succession in Lotic Environments. **Ecology**, 72(5): 1835-1848.

Meyer, E. I. (1989) the relationship between body length parameters and dry mass in running water invertebrates. **Archiv Fur Hydrobiologie**, 117(2): 191-203.

Moog, O. (ed.) (1995). **Fauna Aquatica Austriaca**. Vienna: Wasser Wirtschafts Kataster, Bundesministerium fur land- Forstwirtschaft.

Mosisch, T. D. and Bunn, S. E. (1997) Temporal patterns of rainforest stream epilithic algae in relation to flow-related disturbance. **Aquatic Botany**, 58(2): 181-193.

Munoz, I., Real, M., Guasch, H., et al. (2000) Resource limitation by freshwater snail (Stagnicola vulnerata) grazing pressure: an experimental study. **Archiv Fur Hydrobiologie**, 148(4): 517-532.

Osenberg, C. W., Sarnelle, O. and Cooper, S. D. (1997) Effect size in ecological experiments: the application of biological models in meta-analysis. **The American Naturalist**, 150(6): 798-812.

Paine, R. T. (1980) Food webs - Linkage, interaction strength and community infrastructure -The third Tansley lecture. **Journal of Animal Ecology**, 49(3): 667-685.

Paine, R. T. (1992) Food-Web Analysis through Field Measurement of Per-Capita Interaction Strength. **Nature**, 355(6355): 73-75.

Pardo, I. and Armitage, P. D. (1997) Species assemblages as descriptors of mesohabitats. **Hydrobiologia**, 344: 111-128.

Petersen, J. E. and Englund, G. (2005) Dimensional approaches to designing better experimental ecosystems: a practioner's guide with examples. **Oecologia**, 145: 216-224.

Peterson, C. G. (1996) "Response of algae to natural disturbance". In Stevenson, R. J., Bothwell, M. and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 375-402.

Peterson, C. G., Horton, M. A., Marshall, M. C., et al. (2001) Spatial and temporal variation in the influence of grazing macroinvertebrates on epilithic algae in a montane stream. **Archiv Fur Hydrobiologie**, 153(1): 29-54.

Peterson, C. G., Vormittag, K. A. and Valett, H. M. (1998) Ingestion and digestion of epilithic algae by larval insects in a heavily grazed montane stream. **Freshwater Biology**, 40(4): 607-623.

Poff, N. L., Wellnitz, T. and Monroe, J. B. (2003) Redundancy among three herbivorous insects across an experimental current velocity gradient. **Oecologia**, 134(2): 262-269.

Power, M.E., Tillman, D., Estes, J. A., et al. (1996) Challenges in the quest for keystones. **Bioscience**, 46(8): 609-620.

Prenda, J., Armitage, P. D. and Grayston, A. (1997) Habitat use by the fish assemblages of two chalk streams. **Journal of Fish Biology**, 51(1): 64-79.

Robson, B. J. (1996) Habitat architecture and trophic interaction strength in a river: riffle-scale effects. **Oecologia**, 107: 411-420.

Robson, B. J. and Barmuta, L. A. (1998) The effect of two scales of habitat architecture on benthic grazing in a river. **Freshwater Biology**, 39(2): 207-220.

Rosemond, A. D., Mulholland, P. J. and Elwood, J. W. (1993) Top-Down and Bottom-up Control of Stream Periphyton - Effects of Nutrients and Herbivores. **Ecology**, 74(4): 1264-1280.

Rosenfeld, J. S. (1997) The effect of large macroinvertebrate herbivores on sessile epibenthos in a mountain stream. **Hydrobiologia**, 344: 75-79.

Sala, E. and Graham, M. H. (2002) Community-wide distribution of predator-prey interaction strength in kelp forests. **Proceedings of the National Academy of Sciences of the United States of America**, 99(6): 3678-3683.

Sarnelle, O., Kratz, K. W. and Cooper, S. D. (1993) Effects of an invertebrate grazer on the spatial arrangement of a benthic microhabitat. **Oecologia**, 96: 208-218.

Schindler, D. W. (1998) Replication vs realism: the need for ecosystem-scale experiments. **Ecosystems**, 1: 323-334.

Sole, R. V. and Montoya, J. M. (2001) Complexity and fragility in ecological networks. **Proceedings of the Royal Society of London B Biological Sciences**, 268: 2039-2045.

Steinman, A. D. (1991) Effects of herbivore size and hunger level on periphyton communities. **Journal of phycology**, 27: 54-59.

Steinman, A. D. (1996) "Effects of grazers on freshwater benthic algae". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** Academic Press. pp. 341-366.

Steinman, A. D., McIntire, C. D., Gregory, S. V., et al. (1989) Effects of irradiance and grazing on lotic algal assemblages. **Journal of Phycology**, 25: 478-485.

Steinman, A. D., McIntire, C. D., Gregory, S. V., et al. (1987) Effects of herbivory type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. **Journal of the North American Benthological Society**, 6(3): 175-188.

Tachet, H., Bournaud, M., Richoux, P., et al. (2000) **Invertébrés d'eau douce: systématique, biologie, écologie. Formated for STAR partners by: Usseglio-Polatera P. (2003/04).** Paris: CNRS Editions.

Tall, L., Cloutier, L. and Cattaneo, A. (2006) Grazer-diatom size relationships in an epiphytic community. **Limnology And Oceanography**, 51(2): 1211-1216.

Taylor, B. W., McIntosh, A. R. and Peckarsky, B. L. (2002) Reach-scale manipulations show invertebrate grazers depress algal resources in streams. **Limnology and Oceanography**, 47(3): 893-899.

Thompson, R. M., Hemberg, M., Starzomski, B. M., et al. (2007) Trophic levels and trophic tangles: The prevalence of omnivory in real food webs. **Ecology**, 88(3): 612-617.

Thrush, S. F., Schneider, D. C., Legendre, P., et al. (1997) Scaling-up from experiments to complex ecological systems: where to next? **Journal of Experimental Marine Biology and Ecology**, 216: 245-254.

Towers, D. J., Henderson, I. M. and Veltman, C. J. (1994) Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. **New Zealand journal of marine and freshwater research**, 28: 159-166.

Trunov, N. M., Nikanorov, A. M. and Askelepov, V. N. (1994) Application of experimental ecosystems for researching natural waters: the problem of similarity. **Hydrological, chemical and biological processes of transformation and transport of contaminants in aquatic environments (Procceddings of the Rostov-on-Don symposium May 1993.**, IAHS publication 219: 429-432.

Tuchman, N. C. and Stevenson, R. J. (1991) Effects of Selective Grazing by Snails on Benthic Algal Succession. **Journal of the North American Benthological Society**, 10(4): 430-443.

Wallace, I. D., Wallace, B. and Philipson, G. N. (2003) **Keys to the case-bearing caddis larvae of Britain and Ireland. Scientific publication No. 61.** Ambleside: Freshwater Biological Association.

Wellnitz, T. A. and Ward, J. V. (1998) Does light intensity modify the effect mayfly grazers have on periphyton? **Freshwater Biology**, 39: 135-149.

Wilson, W. G., Osenberg, C. W., Schmitt, R. J., et al. (1999) Complementary foraging behaviours allow coexistence of two consumers. **Ecology**, 80(7): 2358-2372.

Wootton, J. T. (1997) Estimates and tests of per capita interaction strength: diet, abundance, and impact of intertidally foraging birds. **Ecological Monographs**, 67(1): 45-64.

Wootton, J. T. and Emmerson, M. C. (2005) Measurement of interaction strength in nature. **Annual Review of Ecology, Evolution, and Systematics**, 36: 419-444.

Yipp, M. W. (1990) Distribution of the schistosome vector snail *Biomphalaria straminea* (Pulmonata: Planorbiidae) in Hong-Kong. **Journal of Molluscan Studies**, 56: 47-55.

2.7 Tables and figures

Table 2. 1 Mean (+/- 1 SE) conductivity, pH and temperature measured within mesocosms, across experimental blocks.

	Conductivity $\mu S \cdot cm^{-1}$		pH		Temperature º C	
Block 1	528.9	(1.16)	8.3	(0.01)	17.12	(0.02)
Block 2	534.0	(0.58)	8.3	(0.01)	17.17	(0.03)
Block 3	535.1	(0.43)	8.3	(0.02)	17.26	(0.03)
Block 4	535.5	(0.40)	8.3	(0.03)	17.27	(0.02)
Block 5	534.9	(0.64)	8.3	(0.02)	17.30	(0.04)

Table 2. 2 Grazer species used in treatments for trials 1 and 2. Numbers of individuals per replicate are indicated in parentheses.

Trial 1	Trial 2
Control	Control
T. fluviatilis (4)	*P. fontinalis* (3)
V. piscinalis (5)	*R. balthica* (3)
P. antipodarum (7)	*A. vortex* (5)
B. tentaculata (3)	*Planorbis* sp. (6)
G. pulex (5)	*B. buceratus* (7)
S. ignita (5)	*B. scambus* (8)
L. geniculata (4)	*C. luctuosa* grp. (6)
L. volckmari (7)	*L. hirtum* (2)

Table 2. 3 Predominantly grazing and non-grazing categories for herbivore treatments.

Grazing	**Non-grazing**
T. fluviatilis	*B. tentaculata*
V. piscinalis	*C. luctuosa* grp.
P. antipodarum	*G. pulex*
P. fontinalis	*L. geniculata*
R. balthica	*L. hirtum*
A. vortex	*L. volckmari*
Planorbis sp.	
S. ignita	
B. buceratus	
B. scambus	

Table 2. 4 Results of one-way ANOVA for the per capita herbivore impact (dynamic index) on biofilm AFDM and Chlorophyll *a*.

Source of variation	**SS**	**df**	**MS**	**F - Ratio**	***P***
AFDM					
Species	0.29	15	0.02	19.826	< 0.001
Error	0.06	62	0.00		
Total	0.41	78			
Chlorophyll *a*					
Species	2.73	15	0.18	54.434	< 0.001
Error	0.21	62	0.00		
Total	3.35	78			

Table 2. 5 Results showing t-tests for comparisons of the DI for AFDM and chlorophyll *a* against a null value of zero, i.e. no effect, across herbivore treatments. Degrees of freedom for all treatments = 4. Significant differences are highlighted in bold.

		Test Value = 0	
		t	**Sig. (2-tailed)**
DI [AFDM]	*T. fluviatilis*	-21.38	**0.002**
	V. piscinalis	-7.10	**0.002**
	P. antipodarum	-1.56	0.194
	B. tentaculata	-1.29	0.267
	G. pulex	-0.03	0.976
	S. ignita	-2.27	0.086
	L. geniculata	0.93	0.405
	L. volckmari	-0.33	0.759
	P. fontinalis	-1.63	0.179
	R. balthica	-2.56	0.063
	A. vortex	-0.03	0.980
	Planorbis sp.	-2.08	0.106
	B. buceratus	-0.84	0.447
	B. scambus grp.	0.70	0.523
	C. luctuosa grp.	-0.74	0.498
	L. hirtum	-1.16	0.309
DI [Chlorophyll a]	*T. fluviatilis*	-7.49	**0.017**
	V. piscinalis	-5.95	**0.004**
	P. antipodarum	-0.59	0.588
	B. tentaculata	-0.99	0.377
	G. pulex	0.34	0.750
	S. ignita	-2.91	**0.044**
	L. geniculata	-0.03	0.977
	L. volckmari	-1.60	0.184
	P. fontinalis	-2.77	**0.050**
	R. balthica	-4.31	**0.013**
	A. vortex	-2.08	0.106
	Planorbis sp.	-5.22	**0.006**
	B. buceratus	-2.83	**0.047**
	B. scambus grp.	-1.99	0.117
	C. luctuosa grp.	-4.10	**0.015**
	L. hirtum	-3.14	**0.035**

Table 2. 6 Pooled Surber data from July and September 2005 showing numbers of individuals and population densities of experimental animals in the Mill Stream.

Herbivore	**Number (data pooled across surbers and seasons)**	**Density numbers·m^{-2}**
G. pulex	7418	123633
L. geniculata	30	500
P. antipodarum	3573	59550
B. scambus gp.	459	7653
L. volckmari	629	10483
B. tentaculata	1180	19662
A. vortex	11	183
B. buceratus	345	5752
S. ignita	50	833
C. luctuosa gp.	418	6967
V. piscinalis	64	1067
Planorbis sp.	11	183
P. fontinalis	0	0
L. hirtum	317	5283
R. balthica	249	4150
T. fluviatilis	526	8767

Table 2. 7 Results of one-way ANOVA for the population herbivore impact (DI) on biofilm AFDM and Chlorophyll *a*.

Source of variation	**SS**	**df**	**MS**	**F - Ratio**	***P***
AFDM					
Species	23976938	14	1712638	0.457	0.947
Error	217554297	58	3750936		
Total	245624466	73			
Chlorophyll *a*					
Species	222084023	14	15863144	3.882	< 0.001
Error	237033873	58	4086790		
Total	473695792	73			

Figure 2. 1 Schematic diagram showing levels of algal architecture in a typical biofilm, a) adnate layer, b) erect and stalked layer and c) filamentous and overstory layer. Adapted from Steinman (1996), chapter 12, page 345 figure 2.

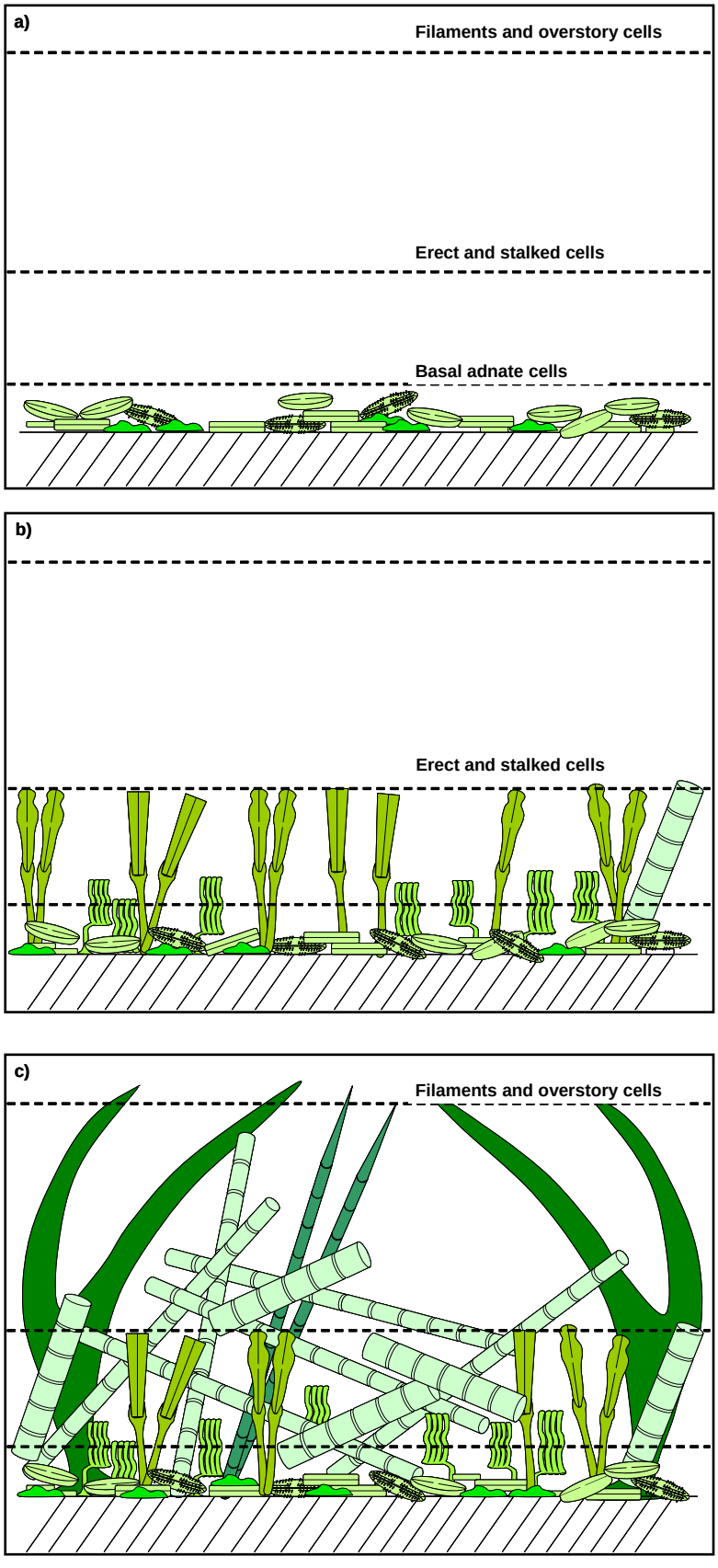


Figure 2. 2 Photographs of the Freshwater Biological Associations Fluvarium, a) upstream – the north channel, to the left is closed, b) downstream.

Figure 2. 3 Diagram representing a single mesocosm viewed from above.

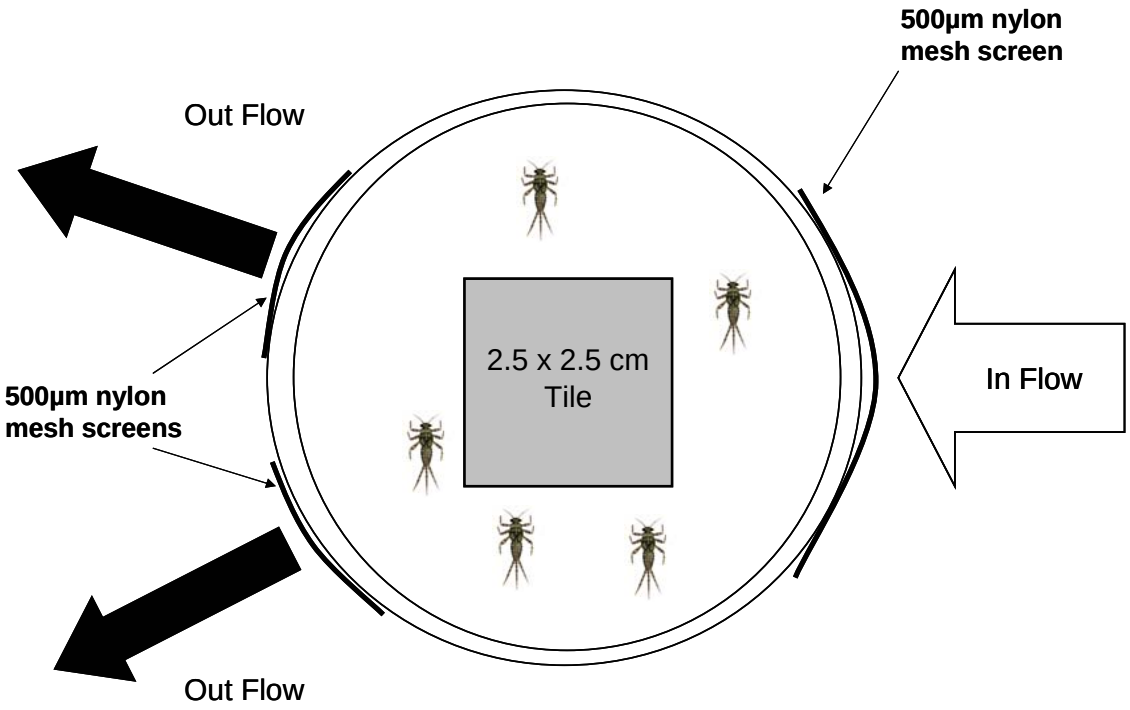
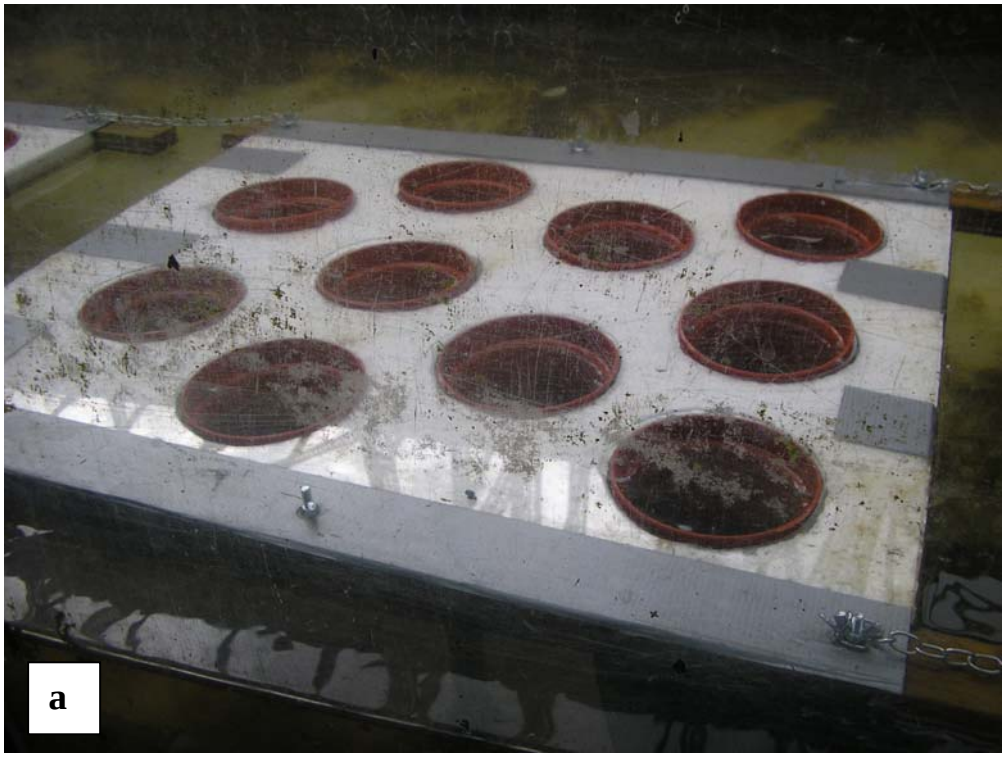


Figure 2. 4 Experimental units in-situ a) seen through glass-sided channel of the fluvarium and b) seen from above.



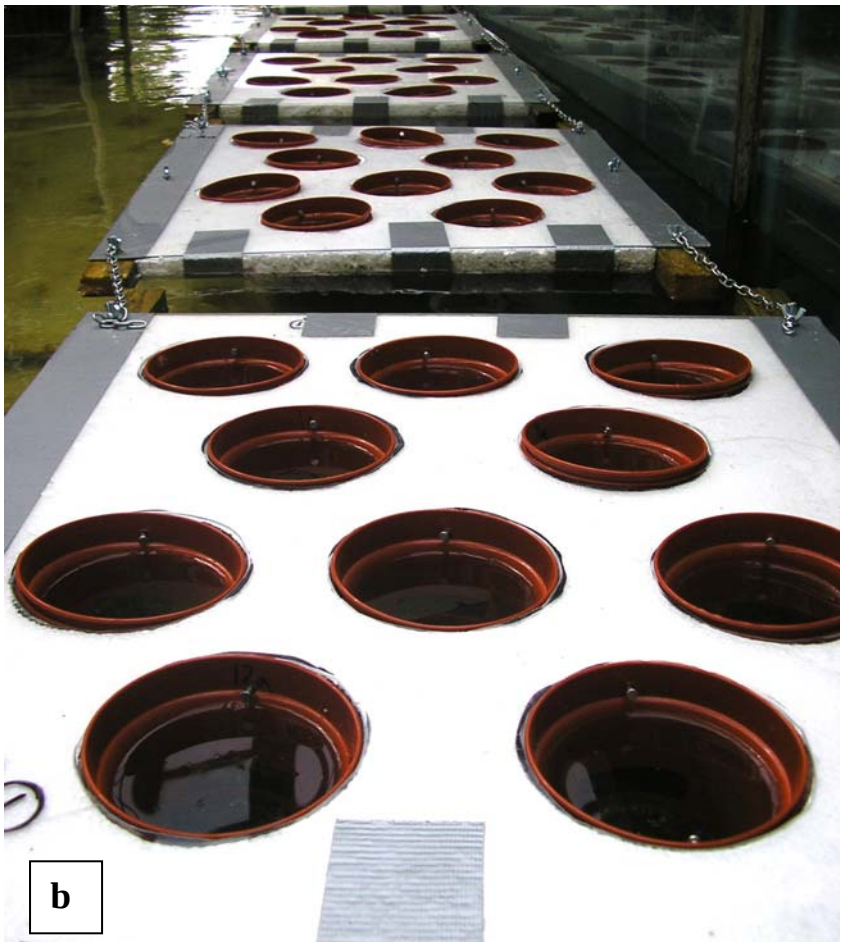


Figure 2.5. Per capita interaction strengths (Dynamic Index) of herbivorous invertebrates on biofilm AFDM and Chlorophyll *a*. Significant differences between AFDM and Chlorophyll *a* within treatments are denoted by different letters, treatments without letters are not different. Tukey's pairwise comparisons show *Theodoxus fluviatilis* has significantly greater impacts than all other herbivore treatments on AFDM and Chlorophyll *a* ($P = < 0.001$ for all comparisons). Means + / - 1 SE are plotted. DI's significantly different from zero (no impact) are denoted with an *****

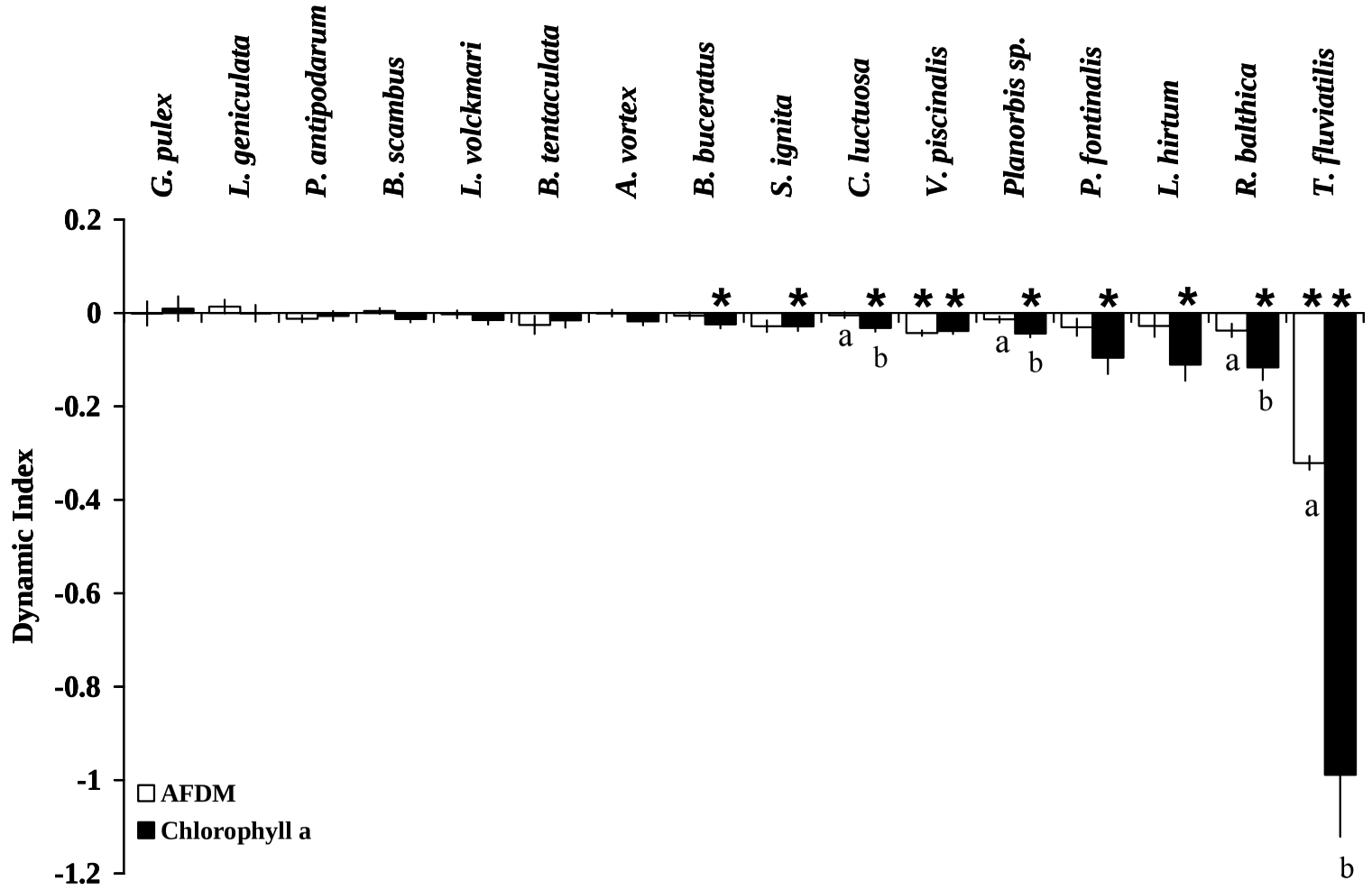


Figure 2.6. Population interaction strengths (Dynamic Index) of herbivorous invertebrates on biofilm AFDM and Chlorophyll *a*. Significant differences between AFDM and Chlorophyll *a* within treatments are denoted by different letters, treatments without letters are not different. Tukey's pairwise comparisons show *Theodoxus fluviatilis* has significantly greater impacts than all other herbivore treatments on Chlorophyll *a*, only ($P = < 0.001$). Means + / - 1 SE are plotted.

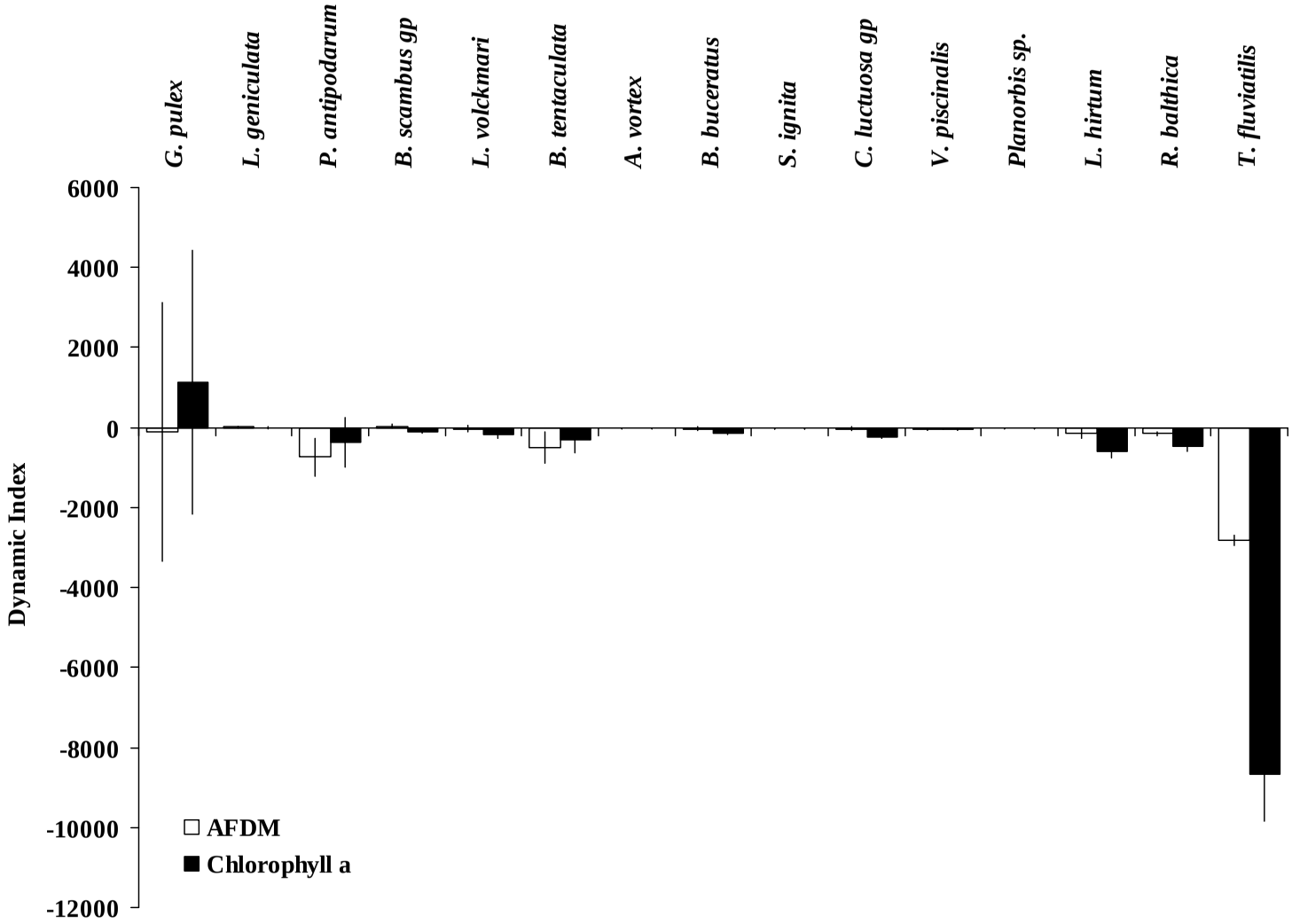


Figure 2.7 Frequency distribution of Dynamic index estimates for (a) all taxa, (b) predominantly grazers and (c) predominantly-non grazers. Arrows indicate where zero interaction lies within the following categories. DI categories: *1* = 0.25 to 0.16; *2* = 0.15 to 0.06; *3* = 0.05 to -0.04; *4* = -0.05 to -0.14; *5* = -0.15 to -0.24; *6* = -0.25 to -0.34; *7* = -0.35 to -0.44; *8* = -0.45 to -0.54; *9* = -0.55 to – 0.64; *10* = -0.65 to -0.74; *11* = -0.75 to -0.84.

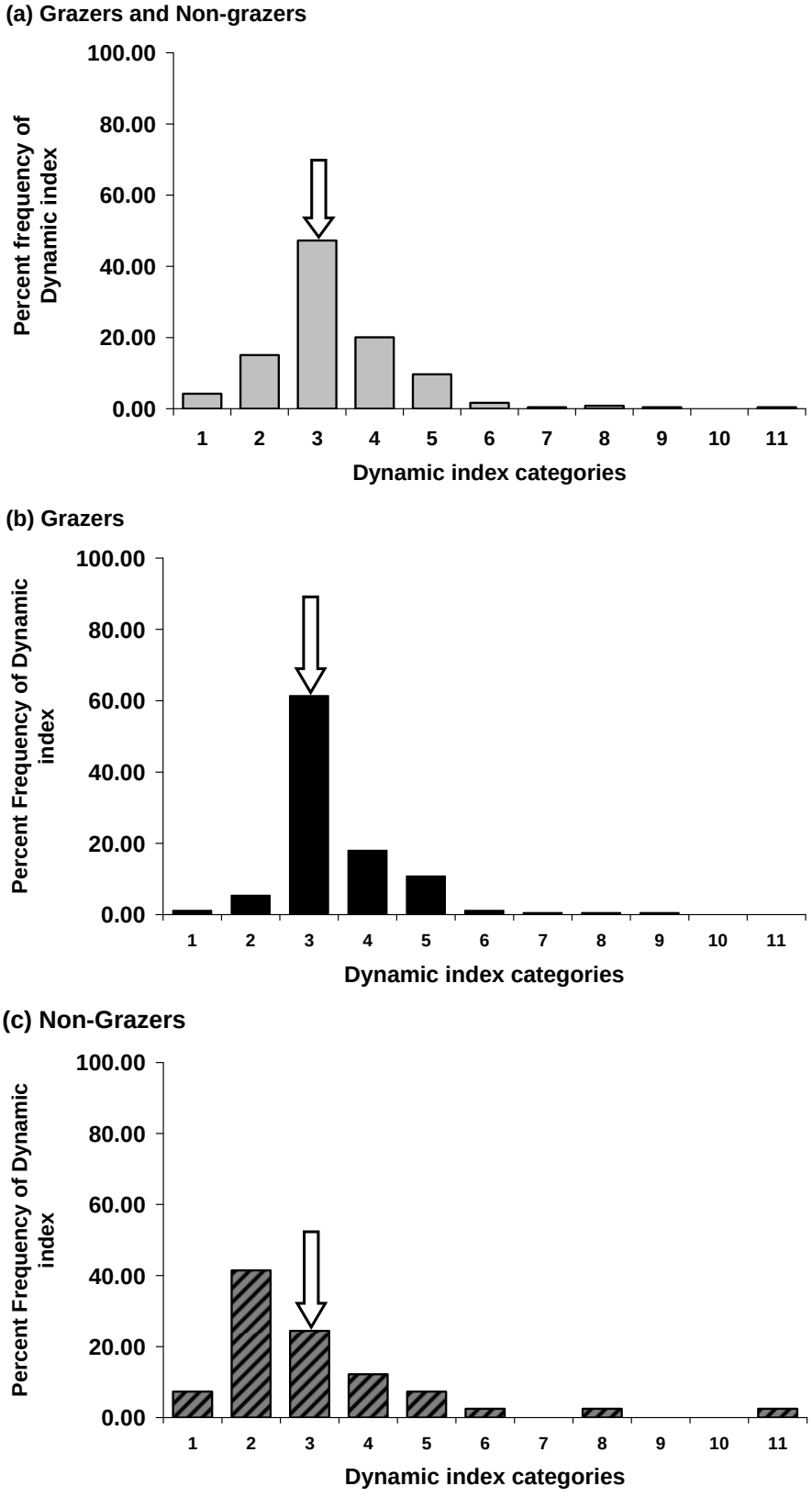


Figure 2.8 Interaction strength (Dynamic Index) gradients for major algal groups across herbivores, (a) Chlorophyta (*Gongrosira incrustans*), (b) Bacillariophyta, (c) Cyanophyta and (d) Chlorophyta (excluding *G. incrustans*).

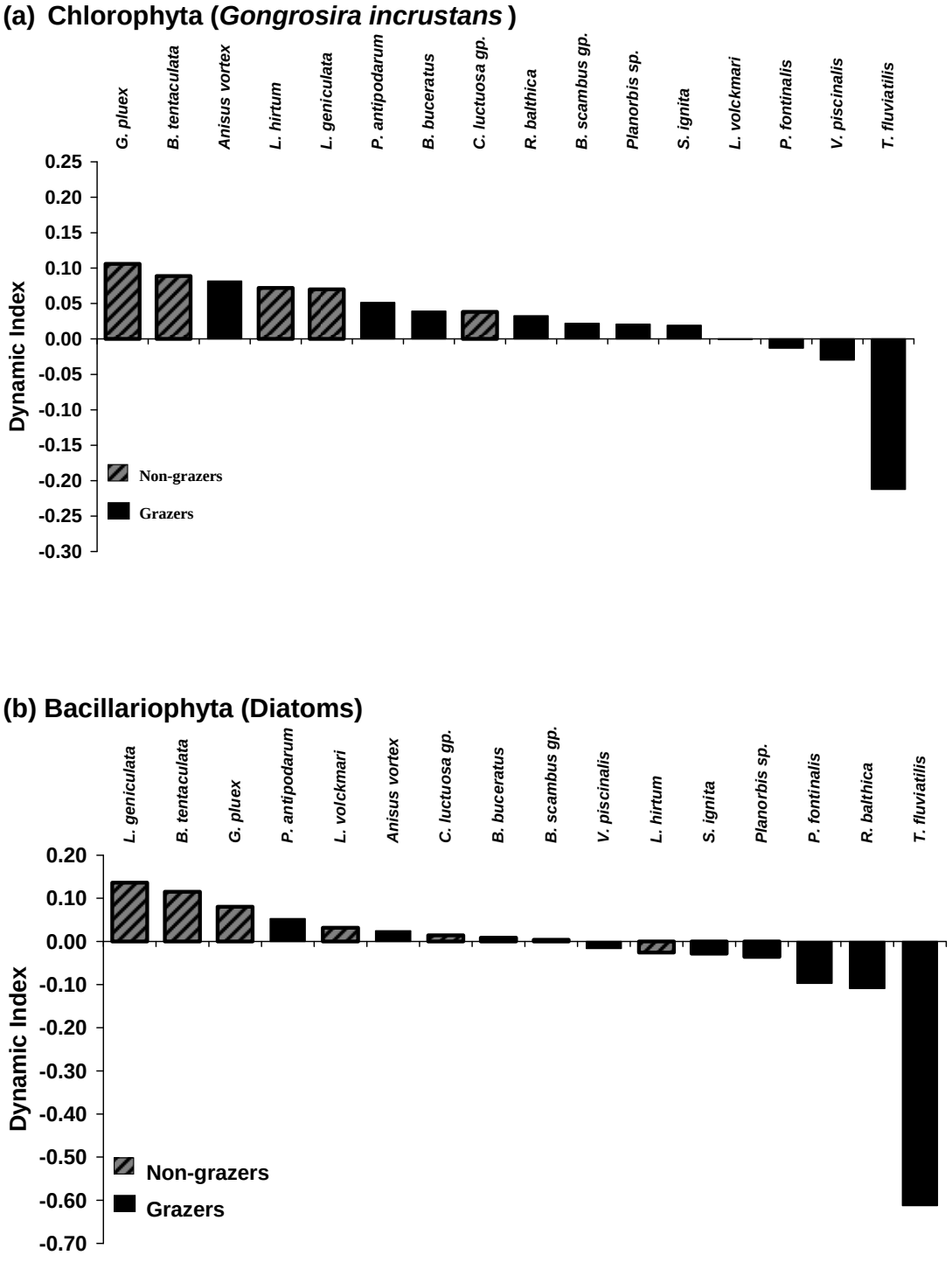
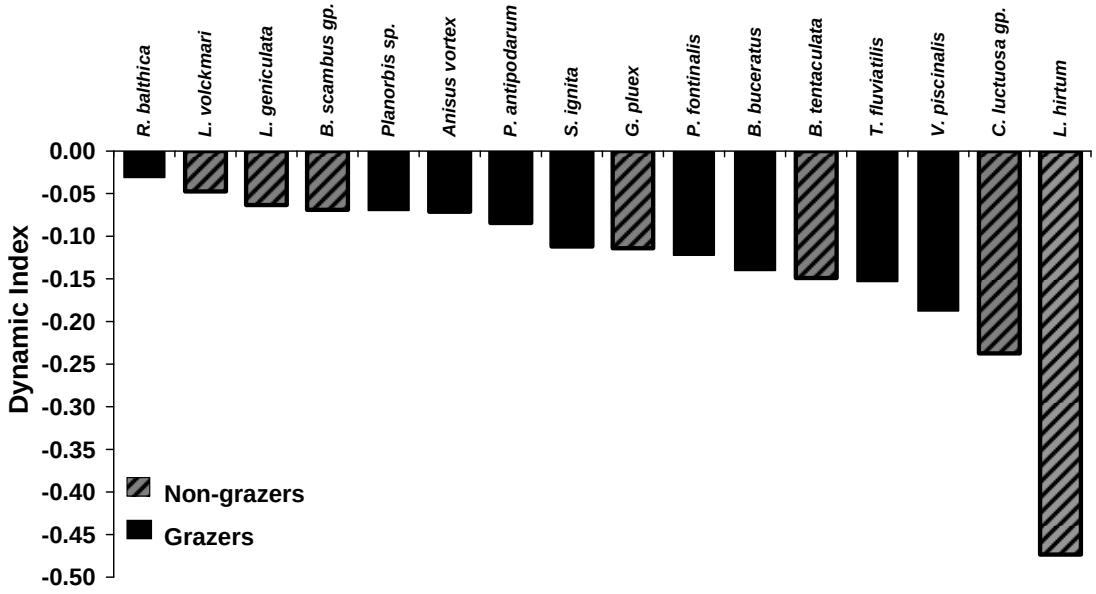


Figure 2.8 Continued.

(c) Cyanophyta (Blue-Green algae)



(d) Chlorophyta (excluding *G. incrustans*)

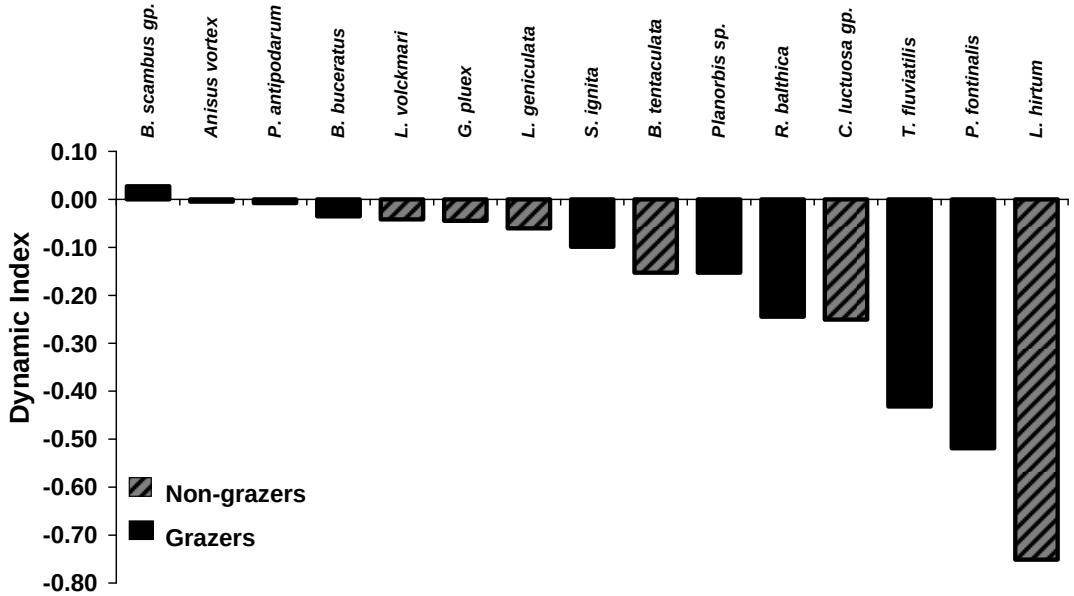


Figure 2.9 Distribution of interaction strength (Dynamic Index) for abundant Diatoms across herbivores, (a) *Melosira varians*, (b) *Achnanthidium minutissimum*, (c) *Cocconeis* spp., (d) *Amphora* spp., and (e) *Nitzschia* spp. (small < 50µm)

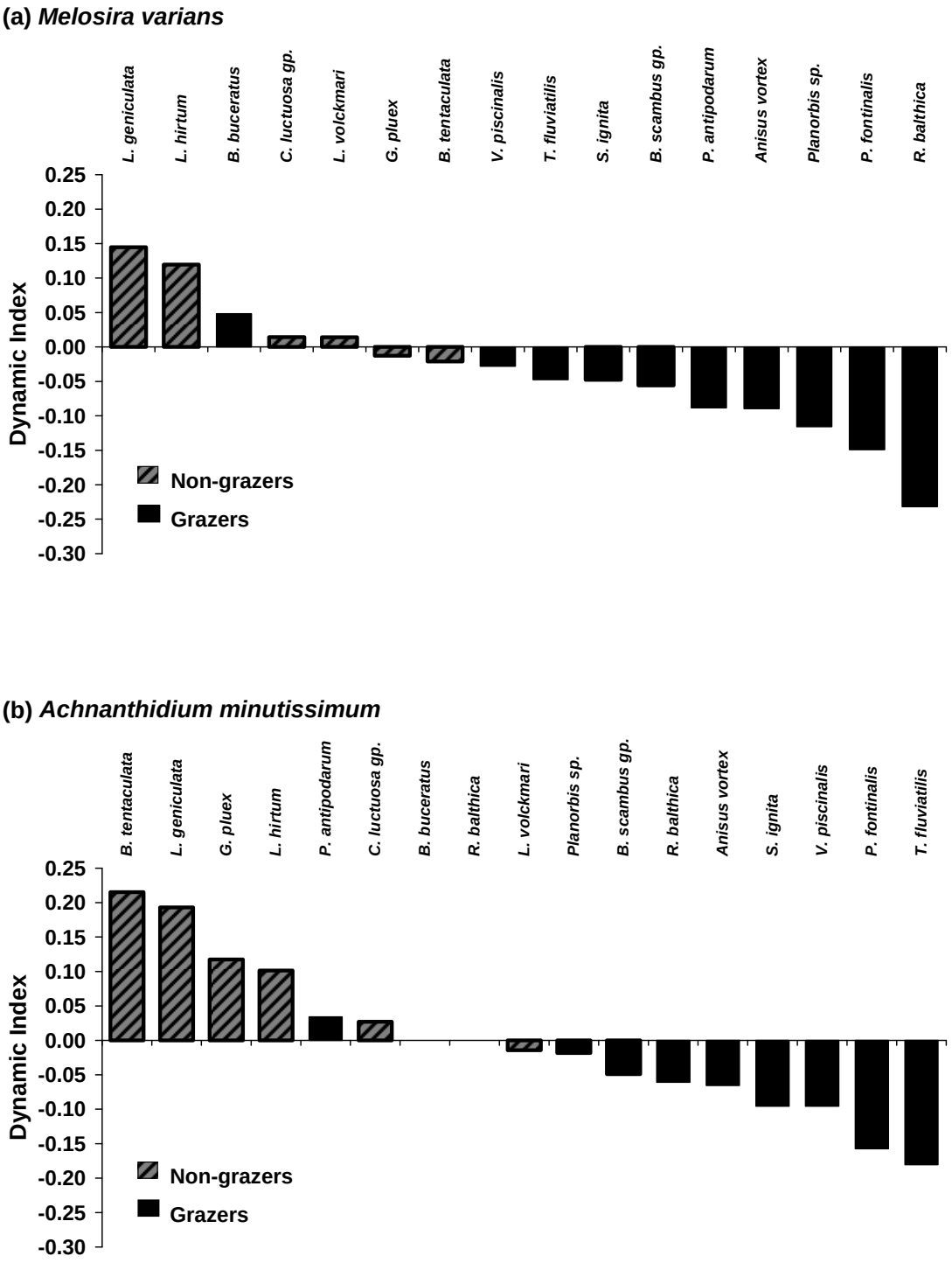
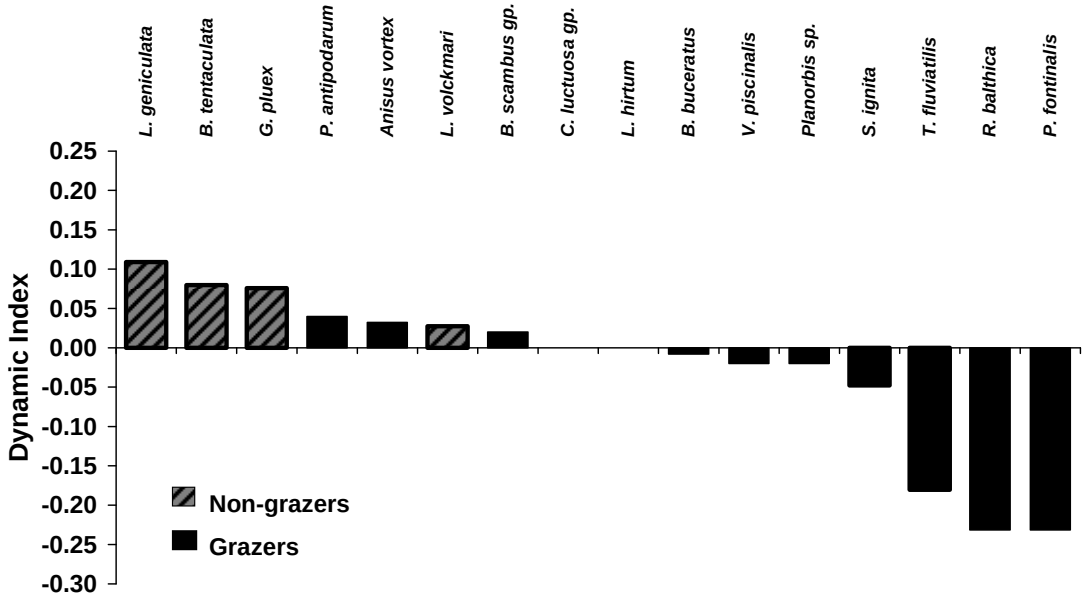


Figure 2.9 continued.

(c) *Cocconeis* ssp.



(d) *Amphora* ssp.

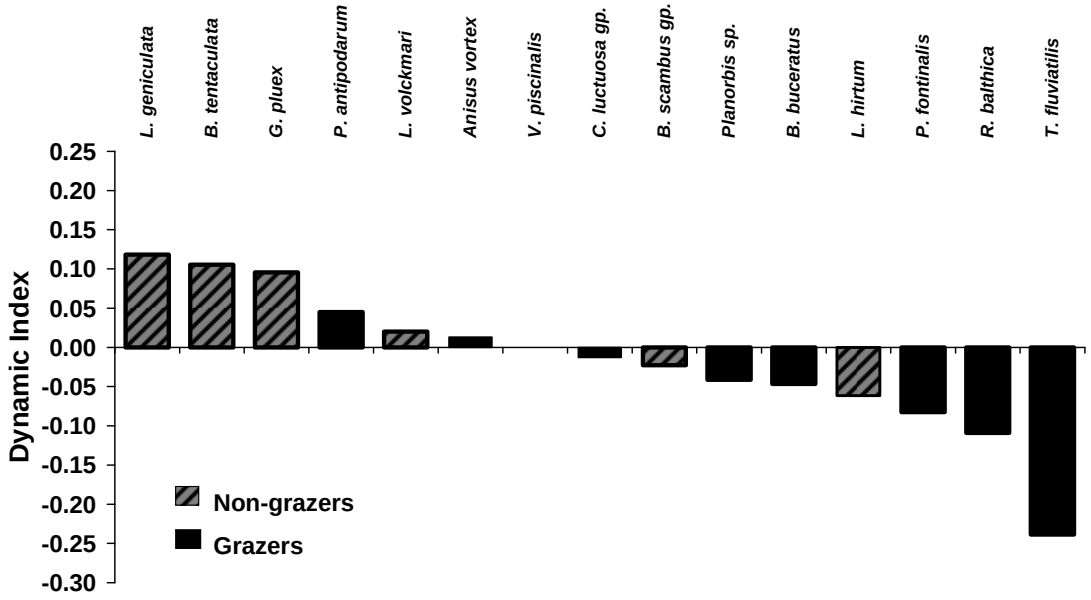
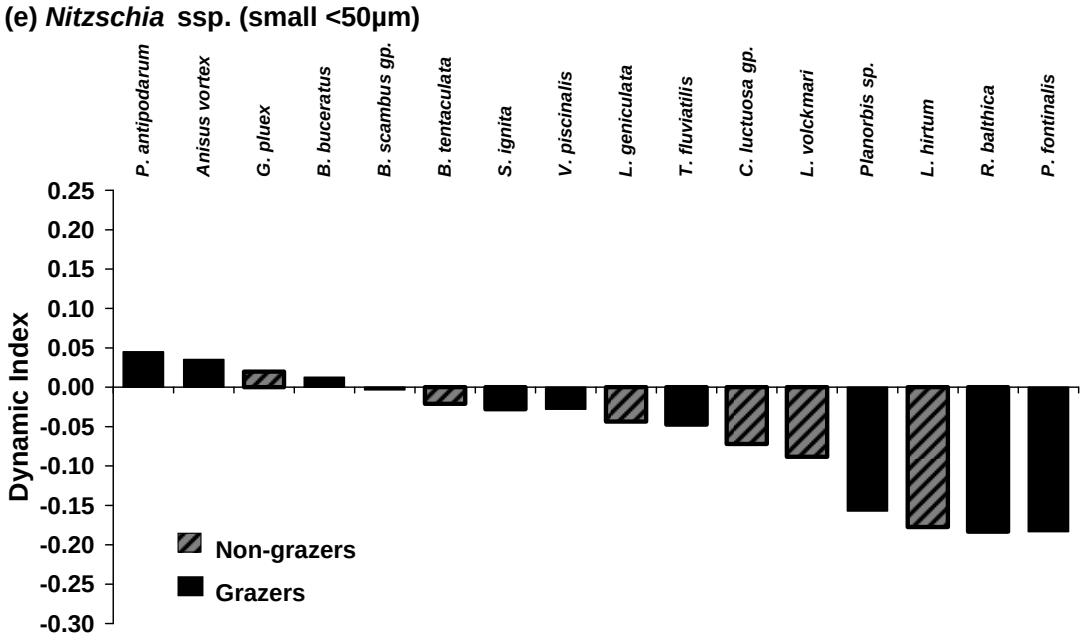


Figure 2.9 continued.



CHAPTER 3 - INTERACTION STRENGTH AND CONTEXT DEPENDENCY - ALGAL MATS VERSUS CRUSTS

3.1 Summary

In chalk streams herbivorous invertebrates forage among patches of benthic algae with contrasting physiognomies. Two algal patch types were observed in a lowland chalk stream in Southern England: 1) filamentous mats dominated by the chain forming diatom *Melosira varians*, and 2) calcareous crusts predominately composed of the green alga *Gongrosira incrustans*. Grazer impacts among resource patches of contrasting structure are likely to vary strongly but have not been widely studied. This study investigated invertebrate grazer effects on biomass (Chlorophyll *a* and AFDM) and taxonomic composition of algae in mat and crust patch types.

Grazing by three gastropod molluscs (*Radix balthica, Theodoxus fluviatilis* and *Bithynia tentaculata*) and three mayfly taxa (*Baetis buceratus* gp, *Baetis scambus* gp and *Serratella ignita*) was compared on the two patches. Grazing on mats was stronger than crusts for some species. The snail *R. balthica* strongly depressed diatom strands but did not affect *G. incrustans*. However, *B. tentaculata* and *T. fluviatilis* significantly reduced crust AFDM, but only weakly. Results suggest that for some herbivores grazing intensity varies depending on the architecture of algae assemblages in habitat patches.

3.2 Introduction

3.2.1 *Food web structure and function: the effect of context dependency*

Food webs based on an interaction matrix dominated by many weak and a few strong links between species are considered to be ecologically stable, and are frequently found by investigators (Chapter 2; Wootton, 1997; Berlow et al., 1999, 2004). However, the strongest interactions are not necessarily species-specific and can shift from one species to another in a context dependent manner. Consequently context-dependent changes in food web structure and function may alter the topography of strong links along spatial-temporal scales (Power et al., 1996). Food web models quantified with context-dependent interaction strengths are thus integral for assessing ecosystem resilience, particularly in the face of increased perturbations from changing climate (Winder and Schindler, 2004; Hooper et al., 2005). They also enhance our current understanding of food web dynamics and permit the development of predictive tools needed for assessing perturbations. For this reason, there is a drive to identify context-dependent factors and ascertain how these affect the structure of links within the network. Interaction strengths are driven in part by differences in the functional responses of species (biotic constraints), and abiotic mechanisms (Jordan, 2002). Biotic drivers, such as trophic cascades alter the topography of strong links by controlling the impact competitively dominant species have at lower levels (Power et al., 1996; Chase, 2003; Miyasaka et al., 2003). Abiotic mechanisms that induce context-dependent interactions include for example, changes in flow regime that causes a switch in the dominant grazer species (Opsahl, Wellnitz and Poff, 2003; Poff,

Wellnitz and Monroe, 2003). Therefore, shifts in environmental conditions expose species with exploitative adaptations, and subsequent shifts in dominant species.

However, evaluation of context-dependent mechanisms is problematic and logistically difficult to complete for complex food webs with thousands of links between organisms (Abrams, 2001). A framework derived from specific food web interactions, such as the interaction between grazing invertebrates and benthic biofilms, may elicit the importance of context-dependent interactions, which can then be applied to whole ecosystems. Resource depression via grazing in freshwater lotic systems has been studied extensively over the last few decades (Feminella and Hawkins, 1995). Investigators have assessed grazer impacts on algal biofilms by altering the abiotic and biotic context of their investigations, for example, varying irradiance (Johnson et al., 1997), nutrient supply (McCormick, 1994; Peterson et al., 2001; Hildebrand, 2003), flow regimes (Mosisch and Bunn, 1997), and grazer density (King-Lotufo et al., 2002) and identity (Rosenfeld, 1997). However, few studies have addressed grazer impacts on structurally distinct algal patches where patch-specific interactions mediate species identity of strong interactors. Grazing is a key function in autochthonous driven streams, where primary production directly links to secondary production through a bottom-up process. Therefore spatial-temporal variation in identity of strong grazers has important implications for primary producers and any subsequent bottom-up food web processes.

3.2.2 *Algal patch types*

Spatial-temporal gradients induced by seasonal and successional mechanisms create mosaics of algal communities varying in assemblage composition (Ledger et al.,

2008). Highly dispersed algal species with rapid turnover and primary production rates initially colonise substrates (McCormick and Stevenson, 1991; Johnson, et al., 1997). As biofilms develop over successional time the community shifts to less motile, sedentary taxa with slower reproductive rates. These late successional species tend to competitively dominate biofilm assemblages (McCormick and Stevenson, 1991; Ledger et al., 2008). However, interruptions to successional processes from localised disturbance may result in divergent patch quality at small spatial scales such as, within the pool / riffle scale (Ledger et al., 2008). Consequently, grazing rates of individual herbivore species are likely to change from patch to patch, in a context-dependent way. For example, Chase et al. (2001) examined the effect of patchy resource distribution on several snail grazer species. Given a gradient of resource patchiness ranging from low (one large substrate) to high (smaller widely dispersed substrates), they found snail species utilised these differently and high patchiness led to a dominance by snails adapted to locating new resources while low patchiness promoted species adapted to local exploitation of resources. However, they did not investigate algal composition as a definition of patchiness, and its impact on specific grazer-algal interactions. Patches exposed to herbivory are likely to incur different grazing impacts depending on the algal species composition and physiognomy. Initial algal colonisers of early successional biofilms have simple physiognomies dominated by low-level diatom species (Johnson et al., 1997). As more species settle, stalked and chain forming diatoms proliferate in the biofilm, forming vast mats of diatom colonies (Ledger et al., 2008), particularly in the chalk streams of Dorset. At this stage the biofilm is more susceptible to grazing and dislodgement than a later successional assemblage with a more complex multi-layered physiognomy. Furthermore, the calcareous crust forming *Gongrosira incrustans* is a dominant element of late

successional algal patches found in the Mill Stream (Dorset). Algal patches dominated by these crusts may be a relatively difficult resource for grazers to utilize (Elser et al., 2005). Consequently, heterogeneous habitats, such as the Mill Stream, where algal patches vary both spatially (Ledger et al., 2008) and temporally (Marker and Casey, 1982) due to successional processes and localised disturbances, offers the grazer guild abundant and diverse resources and promotes species coexistence across patches.

3.2.3 *Grazers*

Grazer effects on benthic algae are frequently dependent on the identity of the grazing species (Steinman, et al., 1987; Feminella and Hawkins, 1995). For example, caddisfly larvae typically exert stronger effects on biofilms when compared to mayfly nymphs (Jacoby, 1987; Hill and Knight, 1988; Feminella and Hawkins, 1995; Poff et al., 2003; McNeely et al., 2007) or snails (Steinman et al., 1987; DeNicola et al., 1990). Nonetheless, significant impacts on biofilm biomass (Liess and Kahlert, 2007), productivity (Steinman, 1996) and assemblage structure (Tuchman and Stevenson, 1991; Underwood et al., 1992; Diaz Villanueva and Modenutti, 2004) has been documented independently for snails and mayflies (Bronmark et al., 1992; Barbee, 2005). Remarkably few studies have concurrently explored impacts on algal biofilms of two taxonomically distinct grazers, such as snails and mayfly nymphs (Feminella and Hawkins, 1995). This is surprising when they often occur together in benthic communities. Furthermore, grazers like snails and mayflies remove algae using morphologically diverse feeding apparatus. Snails graze by rasping algal species from layers of the biofilm using a toothed plate called a radula (Underwood, 1989; Feminella and Hawkins, 1995; Steinman, 1996). Conversely, mayfly larvae mostly

browse loose algal layers that are easily cropped using brush-like mandibles (Hill and Knight, 1987, 1988). Therefore, feeding mode and mouthpart morphologies may offer mechanistic explanations for the variation in grazing effects across snail and mayfly species. However, differences between species within snail and mayfly groups are poorly understood. Examination of grazers within these groups revealed little or no difference in overall impacts across similar species (Liess and Kahlert, 2007; Wilson et al., 1999), but rather showed a divergence in foraging mode facilitating co-existence by partitioning resources (Fuller and Desmond, 1997; Wilson et al., 1999). Therefore, morphologically similar species exerting analogous overall effects on algal biofilms could imply functional redundancy amongst members of the grazer guild.

3.2.4 *Experimental aims*

This investigation was designed to examine the extent to which the strength of interactions between herbivorous macroinvertebrates and benthic algae depend on the identity of the grazing species, and the type of algal patch over which they forage. To this end, a series of comparisons were made quantifying the effects of three snail species and three mayfly species on two algal patch types (mats and crusts). Grazing by taxonomically similar taxa (within snails and mayflies) was compared with that of morphologically distinct grazers (comparing snails and mayflies). It was predicted that algal resource depression would depend on 1) grazer identity, with snails exerting stronger effects than mayfly larvae and 2) algal patch type, with grazers predicted to impact the palatable mats more than the robust crusts; and 3) that species specific effects depend on patch, i.e. there is an interaction between grazer species and patches.

3.3 Methods

3.3.1 *Study site*

Herbivorous invertebrates in the Mill Stream are dominated by gastropod molluscs (predominantly *Radix balthica, Bithynia tentaculata and Theodoxus fluviatilis*) and mayfly larvae (predominantly *Baetis buceratus, Baetis scambus and Seratella ignita*). In deeper reaches macrophyte stands of *Ranunculus* spp. are abundant, but in shaded and riffle areas periphytic biofilms establish freely. Algae develop distinct early and late successional biofilms in this system characterised by loose diatomaceous mats (mainly *Melosira varians*) and calcareous green algal crusts (mainly *Gongrosira incrustans*) respectively (Ledger et al., 2008). Both patch types occur simultaneously in the experimental reach (personal observation).

3.3.2 *Experimental background*

An empirical approach was used to conduct an in-situ mesocosm field experiment in the Mill Stream, a tributary of the River Frome, during the summer of 2005 (50°40'48"N, 2°11'06"W). Mesocosms were installed in the Freshwater Biological Association's fluvarium facility (see chapter two for more detail on experimental mesocosms).

3.3.3 *Algal assemblages and grazers*

Mat and crust patches were cultivated over 90 days on unglazed ceramic tiles (2.5 x 2.5 cm = 6.25 cm^2) incubated in experimental channels fed by the Mill Stream. Tiles left undisturbed for the full period developed characteristic crust assemblages. Conversely, tiles mechanically disturbed by brushing at regular intervals over the 90 days restricted biofilm development to an early successional mat stage. Eight tiles from each biofilm type were sampled before the grazing experiment commenced to compare initial mat and crust algal assemblages. The six grazer species (*Radix balthica, Bithynia tentaculata*, *Theodoxus fluviatilis*, *Baetis buceratus, Baetis Scambus* grp. *and Seratella ignita*) selected were commonly occurring taxa within the two grazer groups (snails and mayflies) and were easily identifiable to species or genus under field conditions. Across these groups feeding mode differed; rasping and scraping snails versus browsing and collecting mayflies, but within groups species feeding morphologies were similar.

3.3.4 *Experimental design*

Each grazer treatment and the ungrazed control were replicated across 5 blocks for both mat and crust patch types. These pair-wise combinations of grazer and patch type (12 treatments x 5 replicates) plus controls (1 replicate of each patch type per block) were assigned to mesocosms arranged in a randomised block design. Individual tiles, randomly selected from mat and crust tiles, were placed in the chambers on a bed of clean sand to provide traction for invertebrates (figure 3.2). Using published and unpublished (Appendix 1) length mass regressions (Mason**,** 1977; Meyer, 1989;

Towers et al., 1994; Burgherr and Meyer, 1997; Benke et al., 1999) grazer densities were adjusted under field conditions to minimise biomass differences across and within groups. Individuals lost through mortality and emergences were replaced daily. After 70 hours grazers and algal biofilms were processed as for chapter 2.

3.3.5 *Data analyses*

One-way ANOVAs ($\alpha = 0.05$) were used to examine differences in biofilm responses (AFDM and Chlorophyll *a*) to grazer treatments and ungrazed controls. Data were found to meet assumptions of normality and homogeneity of variance, using Anderson-Darling and Levene tests respectively ($\alpha = 0.05$). Impacts across grazers was analysed for AFDM and chlorophyll *a* on mats and crusts. Differences in treatment means were compared using Tukey's multiple comparison tests ($\alpha = 0.05$).

Per capita grazer interaction strength on biofilms was calculated using the Dynamic Index: $DI = Ln(N/D)/Yt$ where, N = normal (treatment), D = deleted (ungrazed control), Y = grazer abundance, t = time. Using AFDM and Chlorophyll *a*, and cell count data, per capita impacts (DI) on biofilms were estimated at the algal community and population levels respectively. An ANCOVA ($\alpha = 0.05$), using grazer biomass across replicates as the covariate, was used to discount variability associated with uneven grazer biomass across treatments. In this analysis the covariate did not significantly account for treatment variance ($P = 0.151$ [AFDM], 0.804 [Chl. *a*]) and was excluded from the ANOVA model. Therefore, a two-way ANOVA ($\alpha = 0.05$) was used to test for significant differences in grazer interaction strengths at the algal community level on both patch types, i.e. two factors: grazer (six levels) and patch type (two levels) and their interaction was tested. Tukey's multiple comparisons were

used to test pairwise statistical differences among treatment means. At the algal population level (cell count data) per capita grazer impacts on algal taxa were calculated using the dynamic index. Interaction strength frequency distributions were plotted to analyse grazing impacts across the two algal assemblages.

3.3.6 *Multivariate analysis of algal population data*

Algal community composition of all treatments and controls were analysed using a principal components analysis (PCA). Log_{10} x +1 transformed algal abundance data was used in this analysis. The first two principal components represent most of the explainable variance within the data, and were therefore used in the ordination diagram. Vectors were used to represent major algal groupings.

3.3.7 *Radix balthica and G. incrustans in a natural stream community*

The relationship between *R. balthica* and *G. incrustans* population densities from the Mill Stream survey data (Chapter 2) was tested using a non-parametric Spearman rank correlation.

3.4 Results

3.4.1 *Algal percent composition of patches*

The two patch assemblages were distinct. Before the experiment, mats were dominated by diatoms (97%), mainly *M.* varians, whereas crusts were predominantly consisted of *G. incrustans* (95%) (figure 3.3.a). Algal assemblage structure remained relatively similar to pre-experiment tiles during the course of the experiment (70 hours), i.e. ungrazed controls for each patch type were similar to biofilms at the start of the experiment (figure 3.3.b).

3.4.2 *Absolute treatment effects on algal biomass*

There was a significant effect of grazer treatment on mat AFDM and Chlorophyll *a*, (table 3.1. and figure 3.4) whereas effects on crusts were less consistent, with fewer statistically significant effects on AFDM and Chlorophyll *a* (table 3.1 and figure 3.5). On mats, *R. balthica* significantly reduced algal biomass (AFDM & Chlorophyll *a*) compared to all other grazer treatments and controls (Tukey $P < 0.05$). Compared with controls, *B. tentaculata* and *T. fluviatilis* significantly reduced mat AFDM and Chlorophyll *a* respectively. Only one mayfly species, *S. ignita*, significantly reduced mat biomass relative to controls (AFDM & Chlorophyll *a*). Conversely, grazer treatment impacts on crusts were restricted to significant ($P < 0.05$) but weak resource depression in comparison with controls (table 3.1). *T. fluviatilis* significantly depressed AFDM and Chlorophyll *a*, while *B. tentaculata* lowered AFDM (figure 3.5).

3.4.3 *Dynamic Index – per capita grazer interactions at the community level*

ANOVA demonstrated that interaction strength varied significantly among grazers and algal patch types, and that there was a statistically significant interaction among the main effects for both AFDM and Chlorophyll a, indicating that interaction strength differed significantly among algal patches for some but not all grazers (table 3.2). At the community level, grazer interactions were dominated by a strong and significant impact of *R. balthica* on mats for AFDM and Chlorophyll *a*, which was not replicated on crust patches (figure 3.6). The other grazers interacted weakly with mat algae and *B. tentaculata* and *S. ignita* showed only a minor depression of AFDM. *Bithynia tentaculata*, *S. ignita* and *T. fluviatilis* weakly depressed mat Chlorophyll *a*. Crusts were largely grazer resistant but *T. fluviatilis* exerted a marginally greater impact on crust AFDM and Chlorophyll *a* than other taxa, and *B. tentaculata* affected only AFDM. Contrary to mat assemblages, *R. balthica* exerted the weakest impact on crusts for both AFDM and Chlorophyll *a*. (figure 3.6). Interestingly, *B. tentaculata* on crusts was the only grazer to show a significant disparity between impacts on the two algal biomass estimates. Per capita interaction strength for crust AFDM was significantly stronger than for crust Chlorophyll *a* (figure 3.6.). Overall, mean treatment effects gave a negative response to grazing pressure for each patch type.

3.4.4 *Grazer effects on algal populations*

The distribution of grazer effects across the algal flora of each patch type differed markedly between snail and mayfly larvae grazers (figure 3.7). Most mayfly effects on algae were weakly negative, positive or undetectable. On mats, higher frequencies

of weak negative or null impacts occurred. A similar pattern was observed for crusts with the exception of *S. ignita*, which exerted a greater frequency of weakly positive impacts. Snails, however, exerted stronger interactions (positive and negative) albeit at lower frequencies across algal taxa. *Radix balthica* and *B. tentaculata* both showed a wide distribution of interactions, with mats generally having weak to strong negative impacts and crusts incurring weak to strong positive impacts. *Theodoxus fluviatilis* impacts had almost identical distributions on either patch type, which were predominantly weak negative or zero.

3.4.5 *Ordination of algal community data*

In the algal abundance data set a good proportion of the variance, 44.9 %, was explained by Principal Component 1, and a further 17.1 % was explained by Principal Component 2, in total 66.9 % of the variation. Overall, the ordination explained up to 71.3 % of the variation within the algal abundance data, thus this model was a good fit to the community data (table 3.3). Mat and crust assemblages for all grazer treatments were distinct in ordination space, except *R. balthica* on mats, for which sample points fell between mat and crust assemblages. Crust assemblages were characterised by a *G. incrustans* community with an associated diatom element composed of *Achnanthidium minutissimum*, *Gomphonema parvulum* and *Navicula menisculus*. Mat assemblages were predominantly associated with a diverse diatom community, including *M. varians*, Small *Nitzschia* spp., *and Cocconeis placentula* among others (figure 3.8). Mat and crust assemblages were both influence by Pleurocapsa spp., which was closely associated with axis 2.

3.4.6 *Naturally occurring populations of R. balthica and G. incrustans*

Radix balthica abundance was negatively correlated with *G. incrustans* abundance in the survey data acquired form the natural flora and fauna in the Mill Stream. The correlation was close to statistical significance ($P = 0.065$), and indicates that as *G. incrustans* increases in abundance *R. balthica* abundance decreases (figure 3.9).

3.5 Discussion

3.5.1 *Context-dependency*: *is it important for strong interactions*?

Context-dependent distributions of per capita interaction strengths were clearly evident in this experiment. Grazing of crust and mat biofilms was uniformly weak for all species except for the snail *R. balthica*. However, the strong impact of *R. balthica* was limited to depressing the mat assemblage alone. Consequently, weak interactions were unaffected by contextual constraint, while the strong interaction of *R. balthica* appeared to be highly context-dependent. Evidence supporting context-dependency of strong interactions is overwhelming in all three major biomes; marine, freshwater and terrestrial systems (Power et al., 1996). Vaughn et al (2007) found a strong impact of the filter feeding bivalve *Actinonaias ligamentina* on algal biomass accrual in the summer but not the autumn, which they attributed to increased nitrogen excretion under summer discharge and temperature conditions. Furthermore, of the eight bivalve species they examined *Actinonaias ligamentina* was the only species to show a strong effect, all other species weakly affected algal accrual through both seasons. In the example above, physical parameters of temperature and discharge affected the incidence of the strong link. In an algal-grazer interaction in streamside channels of the upper Colorado River Poff et al. (2003) found an effect of flow on interaction strengths. The authors explored flow regimes, as a contextual factor affecting grazing among two mayfly nymphs and a caddisfly larva. A significant effect of flow on grazing was reported for the caddisfly (*Glossosoma verdona*) but mayfly nymphs (*Baetis bicaudatus* and *Drunella grandis*) were unaffected and removed uniform amounts of biomass across flow regimes. The authors suggested that faster flow

facilitated foraging by *Glossosoma* through impingement of biofilm uprightness. Mayflies on the other hand tolerate changes in flow velocity and were therefore less affected by flow during foraging. However, the factors affecting context-dependency are extensive, and include abiotic and biotic examples (Power et al., 1996) such as changes in; habitat structure (Diehl, 1992 and Power, 1992), prey mortality (Menge et al., 1994) and wave forces (Peterson, 1979) which have all resulted in a switch from strong to weak links. This dynamic ability to switch the topography and structure of strong links within the ecological network may afford species some protection from the risk extinction. In a recent theoretical paper Karlsson et al (2007) describe a model community, influenced by environmental stochasticity, where strong interaction strength increased a species vulnerability to extinction. In such a scenario species conceivably benefit from context-dependent factors, which trigger a decline in interaction strength and reduce any associated extinction risk. Indeed, Karlsson et al's (2007) model demonstrates that variable interaction strengths seem to increase the resilience in community patterns. Consequently, exerting weak impacts on algal crusts may reduce *Radix balthica*'s risk from stochastic environmental events and enhance the community's resilience. A mechanism that assists the transition from strong to weak interactor is proposed below.

3.5.2. *Mat and crust patch types*

Grazer impacts depended on the type of algal patch encountered and its assemblage structure and physiognomy. Physically tough *Gongosira incrustans* crusts impede grazing, while less robust diatom mats appear more susceptible to the impact of grazing. Mat and crust patch types were distinct, divergent in physical organization

and had contrasting physiognomies, which clearly affected grazing interactions for some, but not all species tested in this study. The negative grazer impacts observed on mat biomass and assemblage structure has been well documented for algal communities dominated by diatoms (Underwood et al., 1992; Wellnitz and Ward, 1998; Tall et al., 2006). Nonetheless, reported grazer effects are highly variable, depending on grazer identity, biofilm physiognomy and resident algal species within a particular stream. This is because of the suitability of the mouthparts of different grazers for feeding on different algal patch types and because of the susceptibility of different algae to dislodgement by invertebrates (Feminella and Hawkins, 1995; Steinman, 1996). In contrast, effects of grazing on calcareous crusts dominated by *G. incrustans* have not been investigated. *Gongrosira incrustans* dominated calcareous crusts are widely distributed and often abundant, particularly in limestone catchments, such as the River Frome (Marker and Casey, 1982; Pentecost, 1992). Furthermore, cushions of encrusting *G. incrustans* develop over longer successional time with gradual accrual and continued growth during the winter (Marker and Casey, 1982; Pentecost, 1988). Therefore, crusts are potentially important seasonally and spatially, either as sources of food for grazers or as a dominant substrate for associated epiphytic diatoms, particularly in the winter when diatom abundances are low (Marker and Casey, 1982). However, the ability of grazers to penetrate calcareous biofilms may affect the relative importance of these algae as a consumer resource, for example a study by Elser et al. (2005) found weak effects of grazing snails on calcareous stromatolitic microbial communities in the Rio Mesquites, Mexico, and therefore more data on grazer-crust interactions are required. The difference in grazing pressure between mats and crusts observed in this study merits further

investigation of how these patch types change through time and what implications this has for macro-invertebrate grazers.

3.5.3 *Grazer effects*: *snails and mayflies*

The effect of snails was more varied than for mayfly nymphs. Snails remove and consume algae from the biofilm using a toothed radula (Bronmark, 1989; Underwood et al., 1992). This mode of feeding has been found to exert strong impacts on algal biomass and assemblage structure (Lamberti et al., 1989; Tuchman and Stevenson, 1991; Underwood et al., 1992; Liess and Kahlert, 2007). Snail impacts are often associated with relative depression of the overstory layer and an increase in the relative abundance of understory species (Tuchman and Stevenson, 1991). Despite the strong effect of a single species, comparisons across species have not always yielded differences between them (Brown, 1982; Barnese et al., 1990; Bronmark et al., 1991). Snails are less likely to locate new resource patches via the drift. Consequently, grazing effort may be highly localised for some species. Chase et al. (2001) described a trade-off between the ability of snail species to utilise a resource patch 'area-intensive diggers' and to locate new resources 'area extensive grazers' (Wilson et al., 1999). *Radix balthica*, known as the 'wandering snail' is likely to fall into the area-extensive category. Covering large areas of patch over a relatively short time increases *R. balthica'*s potential to bulldoze and consume loosely attached mats, whilst reducing it's ability to penetrate the tougher calcareous crust assemblages. Conversely, *B. tentaculata* and *T. fluviatilis* fall into the area-intensive category. Localised grazing associated with this type of feeding mode would concentrate impacts at smaller spatial scales within patches, regardless of patch type.

Consequently, resource depression will be locally strong but weak across the whole patch. Furthermore, a stationary feeding mode reduces non-trophic impacts because fewer algae are dislodged by grazers moving across the substrate.

The negative impact of *B. tentaculata* on crust AFDM did not occur with Chlorophyll *a*, suggesting it only removed non-photosynthetic components of the crust biofilm. The feeding mode of *Bithynia tentaculata* combines filtering microalgae from the water column with benthic grazing, therefore in a patch of poor grazing quality this species can switch feeding mode to supplement its diet (Moog, 1995; Tachet et al., 2000). The higher frequency of positive effects on crust taxa and depletion of non-photosynthetic material suggests *B. tentaculata* positively affects crusts, by removal of the senescent biofilm layer (Lamberti and Resh, 1983) and possibly nutrient regeneration from faeces (McCormick and Stevenson, 1991), perhaps by diverting consumptive energy away from grazing and filtering particles that would ordinarily flocculate on the biofilm surface.

On the other hand, the less robust brush-like mandibles of mayflies remove loose upper layers as they skim the substrate (Hill and Knight, 1987), leaving remnants of the biofilm intact and leading to increased algal turnover rates (Steinman, 1996). Generally, the less effective scraping mouthparts of mayfly larvae only weakly depressed algal patches. *Seratella ignita* did have a significant impact on mat biomass estimates, but per capita interaction strength was not significantly different to controls. Mayfly larvae close to emergence feed less than smaller individuals which have several moults to undergo (Steinman, 1996). The individuals used in this study were large, so it is possible feeding rates were low, weakening their impact. Although, Fuller and Desmond (1997) found that mayfly species *Ephemerella subvaria* and *Epeorus* spp. had higher growth rates on diatoms at late instars.

Mayfly larvae in poor quality patches (crusts) may actively locate higher quality patches by entering the drift (Poff et al., 2003). However, mesocosms eliminate drifting. Consequently, mayflies restricted to poor quality patches may develop behavioural responses, for example increasing search time to locate suitable resources, which could reduce grazing efficiency particularly on crusts.

3.5.4 *Conclusions and further work*

.

Consequently, heterogeneous habitats forming mosaics of algal patches alter strengths of grazer interactions across spatial scales in a context-dependent manner. Increased homogeneity of habitat patches, due to disturbance, may therefore have serious implications for grazing invertebrates by reducing the potential for resource partitioning among the grazer guild. Furthermore, the particular grazers affected will depend on the nature of the perturbation (Ledger et al., 2006). Restriction of algal succession via mechanical disturbance, such as increased spate events, may lead to higher spatial distributions of mat assemblages and could increase competitive dominance by *R. balthica*. Alternatively, under a regime of sporadic dewatering events where mats are succeeded by drought-resistant crusts (Ledger et al., 2008), other snail species may replace *R. balthica* as the dominant grazer. Interestingly, invertebrate survey data collected from the Mill Stream during the experimental season seems to partly concur with this suggestion, in so much as *R. balthica* densities appear to decline as *G. incrustans* numbers increase. Although not a significant relationship, this pattern warrants further investigation.

It is essential that food web modellers incorporate context-dependent parameters in their models if these are to attempt to represent highly heterogeneous natural systems.

Abiotic and biotic gradients appear to promote context-dependency within complex habitats (Opsahl et al., 2003 and Poff et al., 2003). These habitats are often referred to as facilitating species coexistence through resource partitioning that prohibits dominance by individual species performing a particular process (Poff et al., 2003 and Vaughn et al., 2007), and thus promotes context-dependent relationships across such gradients (Poff et al., 2003). Context-dependency is a common phenomenon across marine, freshwater and terrestrial systems (Power et al., 1996), but research needs to explicitly target a range of biotic and abiotic gradients to determine exactly how common context-dependent interactions are in complex food webs.

Assessing per capita interaction strengths using population level data can enhance the overall signal detected by community analysis.

Identification of grazer impacts caused by direct trophic interactions versus indirect mechanical dislodgement were beyond the scope of this study. However, this subject does merit further analysis, as the consequences for the food web as a whole differ. Grazers moving over substrates are likely to dislodge certain components of biofilms, which is therefore not immediately converted to secondary production but transported lower down the stream system. Hence it is important to quantify actual consumption, for example through gut content analysis of grazers, although this will not provide fully accurate consumption data (due to differential digestibility of algal cells).

Maintaining constant grazer body size for this experiment across groups would, if it were possible, have made a comparison of grazers at a specific stage of their life history. However, during a grazer's life history its potential for consuming algae will fluctuate (Fuller and Desmond, 1997). For example, small mayflies that are at less risk from predation may forage for algae more frequently, than larger individuals that are restricted to diel foraging in the presence of fish predators (Culp and Scrimgeour,

1993). Acting as a proxy for life history traits, the next step is to quantify links by assessing grazer body size, and this is studied in chapter 4.

3.6 References

Abrams, P. A. (2001) Describing and quantifying interspecific interactions: a commentary on recent approaches. **Oikos**, 94: 209-218.

Barbee, N. C. (2005) Grazing insects reduce algal biomass in a neotropical stream. **Hydrobiologia**, 532: 153-165.

Barnese, L. E., Lowe, R. L. and Hunter, R. D. (1990) Comparative grazing efficiency of pulmonate and prosobranch snails. **Journal of the North American Benthological Society**, 9(1): 35-44.

Benke, A. C., Huryn, A. D., Smock, L. A., et al. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. **Journal of the North American Benthological Society**, 18(3): 308-343.

Berlow, E. L., Navarrete, S. A., Briggs, C. J., et al. (1999) Quantifying variation in the strengths of species interactions. **Ecology**, 80(7): 2206-2224.

Berlow, E. L., Neutel, A. M., Cohen, J. E., et al. (2004) Interaction strengths in food webs: issues and opportunities. **Journal of Animal Ecology**, 73(3): 585-598.

Bronmark, C. (1989) Interactions between epiphytes, macrophytes and freshwater snails: a review. **Journal of Molluscan Studies**, 55: 299-311.

Bronmark, C., Klosiewski, S. P. and Stein, R. A. (1992) Indirect effects of predation in a freshwater benthic food chain. **Ecology**, 73(5): 1662-1674.

Bronmark, C., Rundle, S. D. and Erlandsson, A. (1991) Interactions Between Fresh-Water Snails And Tadpoles - Competition And Facilitation. **Oecologia**, 87(1): 8-18.

Brown, K. M. (1982) Resource Overlap And Competition In Pond Snails - An Experimental-Analysis. **Ecology**, 63(2): 412-422.

Burgherr, P. and Meyer, E. I. (1997) Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. **Archiv Fur Hydrobiologie**, 139(1): 101-112.

Chase, J. M., Wilson, W. G. and Richards, S. A. (2001) Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. **Ecology Letters**, 4: 304-312.

Chase, J. M. (2003) Strong and weak trophic cascades along a productivity gradient. **Oikos**, 101: 187-195.

Culp, J. M. and Scrimgeour, G. J. (1993) Size-Dependent Diel Foraging Periodicity of a Mayfly Grazer in Streams with and without Fish. **Oikos**, 68(2): 242-250.

DeNicola, D. M., McIntire, C. D., Lamberti, G. A., et al. (1990) Temporal patterns of grazer-periphyton interactions in laboratory streams. **Freshwater Biology**, 23: 475-489.

Diaz Villanueva, V. and Modenutti, B. (2004) Experimental analysis of grazing by the mayfly Meridialaris chiloeensis on different successional stages of stream periphyton. **International Review of Hydrobiology**, 89(3): 263-277.

Diehl, S. (1992) Fish Predation And Benthic Community Structure - The Role Of Omnivory And Habitat Complexity. **Ecology**, 73(5): 1646-1661.

Elser, J. J., Schampel, J. H., Kyle, M., et al. (2005) Response of grazing snails to phosphorus enrichment of modern stromatolitic microbial communities. **Freshwater Biology**, 50: 1826-1835.

Feminella, J. W. and Hawkins, C. P. (1995) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. **Journal of the North American Benthological Society**, 14(4): 465-509.

Fuller, R. L. and Desmond, C. (1997) Influence of food type on the growth of early and late instars of three mayfly (Ephemeroptera) species. **Archiv Fur Hydrobiologie**, 141(1): 1-11.

Hill, W. R. and Knight, A. W. (1987) Experimental analysis of the grazing interaction between a mayfly and stream algae. **Ecology**, 68(6): 1955-1965.

Hill, W. R. and Knight, A. W. (1988) Concurrent Grazing Effects of 2 Stream Insects on Periphyton. **Limnology and Oceanography**, 33(1): 15-26.

Hillebrand, H. (2003) Opposing effects of grazing and nutrients on diversity. **Oikos**, 100(3): 592-600.

Hooper, D. U., Chaplin, F. S., Ewel, J. J., et al. (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. **Ecological Monographs**, 75(1): 3-35.

Jacoby, J. M. (1987) Alterations in periphyton characteristics due to grazing in a cascade foothill stream. **Freshwater Biology**, 18(3): 495-508.

Johnson, R. E., Tuchman, N. C. and Peterson, C. G. (1997) Changes in the vertical microdistribution of diatoms within a developing periphyton mat. **Journal of the North American Benthological Society**, 16(3): 503-519.

Jordan, F. (2002) Searching for keystones in ecological networks. **Oikos**, 99(3): 607-612.

Karlsson, P., Jonsson, T. and Jonsson, A. (2007) Food web structure and interaction strength pave the way for vulnerability to extinction. **Journal Of Theoretical Biology**, 249(1): 77-92.

Kelly, M. (2000) Identification of common benthic diatoms in rivers. **Field studies**, 9: 583-700.

King-Lotufo, E. C., Brown, K. M. and Carman, K. R. (2002) The influence of periphyton biomass and density on grazing in Physella virgata. **Hydrobiologia**, 482(1-3): 23-29.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., et al. (1989) Productive Capacity of Periphyton as a Determinant of Plant Herbivore Interactions in Streams. **Ecology**, 70(6): 1840-1856.

Lamberti, G. A. and Resh, V. H. (1983) Stream periphyton and insect herbivores - An experimental study of grazing by a caddisfly population. **Ecology**, 64(5): 1124-1135.

Ledger, M. E., Harris, R. M. L., Armitage, P. D., et al. (2008) Disturbance, patchiness and coexistence in freshwater algal communities. **Oecologia**, 155: 609-819.

Ledger, M. E., Harris, R. M. L., Milner, A. M., et al. (2006) Disturbance, biological legacies and community development in stream mesocosms. **Oecologia**, 148: 682-691.

Liess, A. and Kahlert, M. (2007) Gastropod grazers and nutrients, but not light, interact in determining periphytic algal diversity. **Oecologia**, 152(1): 101-111.

Marker, A. F. H. and Casey, H. (1982) The population and production dynamics of benthic algae in an artificial recirculating hard-water stream. **Phil. Trans. R. Soc. Lond. B**, 298: 265-308.

Mason, C. F. (1977) Populations and production of benthic animals in two contrasting shallow lakes in Norfolk. **Journal of Animal Ecology**, 46: 147-172.

McCormick, P. V. (1994) Evaluating the Multiple Mechanisms Underlying Herbivore Algal Interactions in Streams. **Hydrobiologia**, 291(1): 47-59.

McCormick, P. V. and Stevenson, R. J. (1991) Mechanisms of Benthic Algal Succession in Lotic Environments. **Ecology**, 72(5): 1835-1848.

McNeely, C., Finlay, J. C. and Power, M. E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. **Ecology**, 88(2): 391-401.

Menge, B. A., Berlow, E. L., Blanchette, C. A., et al. (1994) The Keystone Species Concept - Variation in Interaction Strength in a Rocky Intertidal Habitat. **Ecological Monographs**, 64(3): 249-286.

Meyer, E. I. (1989) the relationship between body length parameters and dry mass in running water invertebrates. **Archiv Fur Hydrobiologie**, 117(2): 191-203.

Miyasaka, H., Genkai-Kato, M., Kuhara, N., et al. (2003) Predatory fish impact on competition between stream insect grazers: a consideration of behaviorally- and density-mediated effects on an apparent coexistence pattern. **Oikos**, 101: 511-520.

Moog, O. (ed.) (1995). **Fauna Aquatica Austriaca**. Vienna: Wasser Wirtschafts Kataster, Bundesministerium fur land- Forstwirtschaft.

Mosisch, T. D. and Bunn, S. E. (1997) Temporal patterns of rainforest stream epilithic algae in relation to flow-related disturbance. **Aquatic Botany**, 58(2): 181-193.

Opsahl, R. W., Wellnitz, T. and Poff, N. L. (2003) Current velocity and invertebrate grazing regulate stream algae: results of an in situ electrical exclusion. **Hydrobiologia**, 499(1-3): 135-145.

Pentecost, A. (1988) Observations on growth rates and calcium carbonate deposition in the green alga *Gongrosira*. **New Phytologist**, 110: 249-253.

Pentecost, A. (1992) Growth and distribution of endolithic algae in some North Yorkshire streams (UK). **British Phycological Journal**, 27: 145-151.

Peterson, C. H. (1979) Importance Of Predation And Competition In Organizing The Inter-Tidal Epifaunal Communities Of Barnegat-Inlet, New-Jersey. **Oecologia**, 39(1): 1-24.

Peterson, C. G., Horton, M. A., Marshall, M. C., et al. (2001) Spatial and temporal variation in the influence of grazing macroinvertebrates on epilithic algae in a montane stream. **Archiv Fur Hydrobiologie**, 153(1): 29-54.

Poff, N. L., Wellnitz, T. and Monroe, J. B. (2003) Redundancy among three herbivorous insects across an experimental current velocity gradient. **Oecologia**, 134(2): 262-269.

Power, M. E., Marks, J. C. and Parker, M. S. (1992) Variation in the vulnerability of prey to different predators: community-level consequences. **Ecology**, 73: 2218-2223.

Power, M. E., Tilman, D., Estes, J. A., et al. (1996) Challenges in the quest for keystones. Identifying keystones is difficult-but essential to understanding how loss of a species will affect ecosystems. **Bioscience**, 46(8): 609-620.
Rosenfeld, J. S. (1997) The effect of large macroinvertebrate herbivores on sessile epibenthos in a mountain stream. **Hydrobiologia**, 344: 75-79.

Steinman, A. D. and Lamberti, G. A. (1996) "Biomass and pigments of benthic algae". In Hauer, F. R. and Lamberti, G. A. (ed.) **Methods in stream ecology.** London, Academic press. pp. 295-313.

Steinman, A. D., McIntire, C. D., Gregory, S. V., et al. (1987) Effects of herbivory type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. **Journal of the North American Benthological Society**, 6(3): 175-188.

Tachet, H., Bournaud, M., Richoux, P., et al. (2000) **Invertébrés d'eau douce: systématique, biologie, écologie. Formated for STAR partners by: Usseglio-Polatera P. (2003/04).** Paris: CNRS Editions.

Tall, L., Cloutier, L. and Cattaneo, A. (2006) Grazer-diatom size relationships in an epiphytic community. **Limnology And Oceanography**, 51(2): 1211-1216.

Towers, D. J., Henderson, I. M. and Veltman, C. J. (1994) Predicting dry weight of New Zealand aquatic macroinvertebrates from linear dimensions. **New Zealand journal of marine and freshwater research**, 28: 159-166.

Tuchman, N. C. and Stevenson, R. J. (1991) Effects of Selective Grazing by Snails on Benthic Algal Succession. **Journal of the North American Benthological Society**, 10(4): 430-443.

Underwood, G. J. C. (1989) The influence of grazing by freshwater pulmonate snails on the population sizes of particular epiphytic algae. **Proceedings of the 10th International Malacological congress**.

Underwood, G. J. C., Thomas, J. D. and Baker, J. H. (1992) An experimental investigation of interactions in snail-macrophyte-epiphyte systems. **Oecologia**, 91: 587-595.

Vaughn, C. C., Spooner, D. E. and Galbraith, H. S. (2007) Context-dependent species identity effects within a functional group of filter-feeding bivalves. **Ecology**, 88(7): 1654-1662.

Wellnitz, T. A. and Ward, J. V. (1998) Does light intensity modify the effect mayfly grazers have on periphyton? **Freshwater Biology**, 39: 135-149.

Wilson, W. G., Osenberg, C. W., Schmitt, R. J., et al. (1999) Complementary foraging behaviors allow coexistence of two consumers. **Ecology**, 80(7): 2358-2372.

Winder, M. and Schindler, D. E. (2004) Climate change uncouples trophic interactions in an aquatic ecosystem. **Ecology**, 85(8): 2100-2106.

Wootton, J. T. (1997) Estimates and tests of per capita interaction strength: diet, abundance, and impact of intertidally foraging birds. **Ecological Monographs**, 67(1): 45-64.

3.7 Tables and figures

Table 3. 1 Results of ANOVA on differences in mean algal biomass estimates for grazer treatments on Mat and Crust patches.

Source of variation	SS	df	MS	F - Ratio	*P*
Mat [AFDM]					
Species	298195382	6	49699230	22.504	< 0.001
Error	61837148	28	2208470		
Total	2916014653	35			
Mat [Chlorophyll *a*]					
Species	29592	6	4932	20.173	< 0.001
Error	6846	28	244		
Total	252366	35			
Crust [AFDM]					
Species	17375457	6	2895909	4.740	0.002
Error	17108435	28	611016		
Total	694153544	35			
Crust [Chlorophyll *a*]					
Species	423	6	70.49	2.626	0.038
Error	752	28	26.85		
Total	19446	35			

Table 3. 2 Results of ANOVA testing differences in grazer estimated interactions for two algal biomass estimates, AFDM and Chlorophyll *a*.

Source of variation	**SS**	**df**	**MS**	**F - Ratio**	***P***
DI [AFDM]					
Grazer	0.064	5	0.013	25.71	< 0.001
Patch	0.009	1	0.009	19.16	< 0.001
Grazer x Patch	0.072	5	0.014	28.87	< 0.001
Error	0.024	48	0.001		
Total	0.170	59			
DI [Chlorophyll *a*]					
Grazer	0.113	5	0.023	34.64	< 0.001
Patch	0.033	1	0.033	50.14	< 0.001
Grazer x Patch	0.123	5	0.025	37.65	< 0.001
Error	0.031	48	0.001		
Total	0.300	59			

Table 3. 3 Results of Principal components analysis. Eigenvalues for axes 1 to 4 and cumulative percentage variance of species data represented on each axis. For a) Mat and Crusts combined; b) Mats alone; c) Crusts alone

a)

Axes	1	2	3	4	Total variance
Eigenvalues	0.449	0.171	0.05	0.044	0.714
Cumulative percentage variance of species data	44.9	61.9	66.9	71.3	

b)

Axes	1	2	3	4	Total variance
Eigenvalues	0.384	0.101	0.089	0.064	0.638
Cumulative percentage variance of species data	38.4	48.5	57.4	63.8	

c)

Axes	1	2	3	4	Total variance
Eigenvalues	0.266	0.174	0.111	0.077	0.628
Cumulative percentage variance of species data	26.6	44.0	55.1	62.8	

Figure 3. 1 One experimental unit in-situ a) seen through glass-sided channel of the fluvarium and b) seen from above.

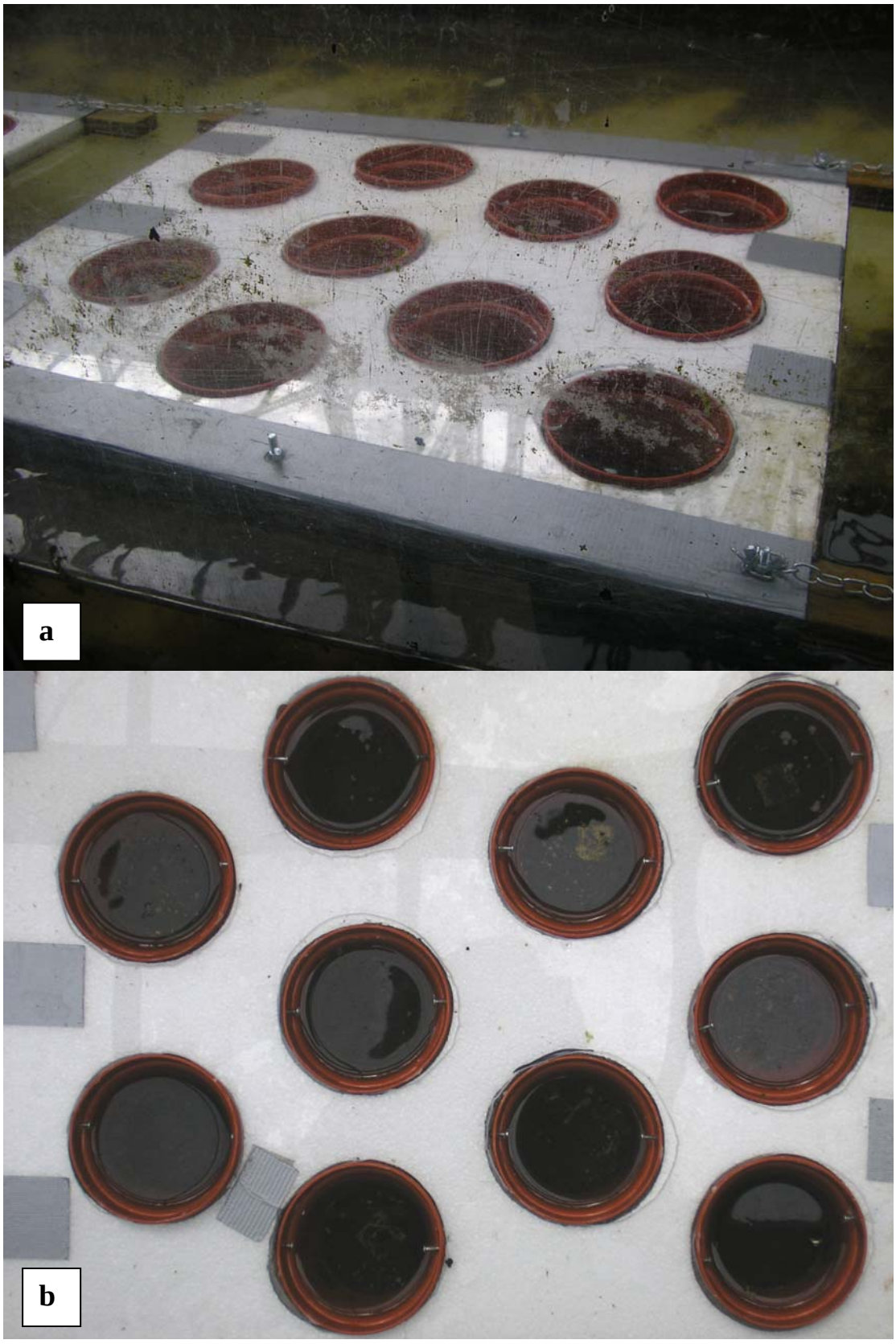


Figure 3. 2 Algal patch types in experimental mesocosms, a) *Theodoxus fluviatilis* on a mat tile and b) *Radix balthica* on a crust tile.

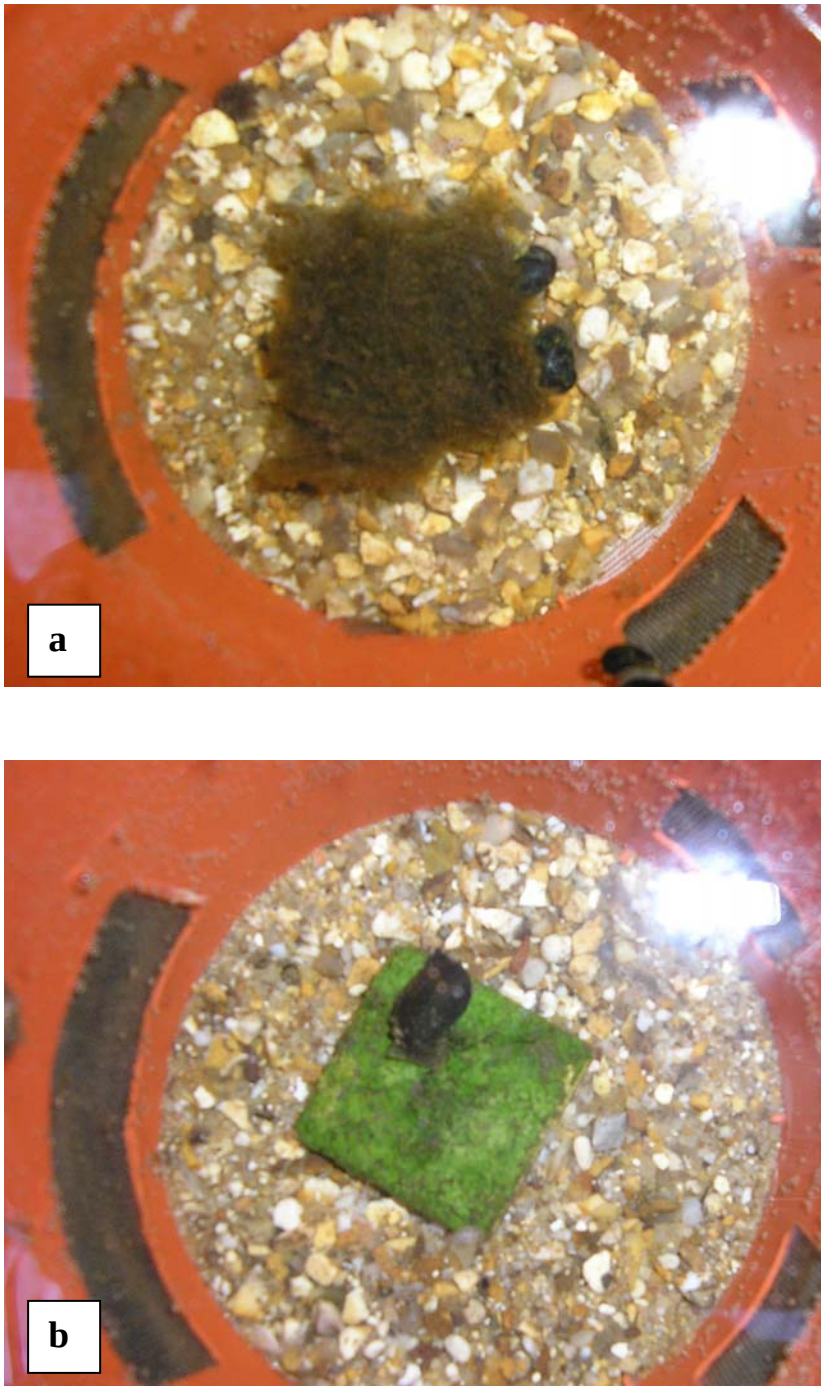


Figure 3. 3 Mean percent composition of algal taxa (excluding taxa with an overall abundance of < 5 %) for mats and crusts, (a) prior to the start and (b) ungrazed controls at the end of the experiment.

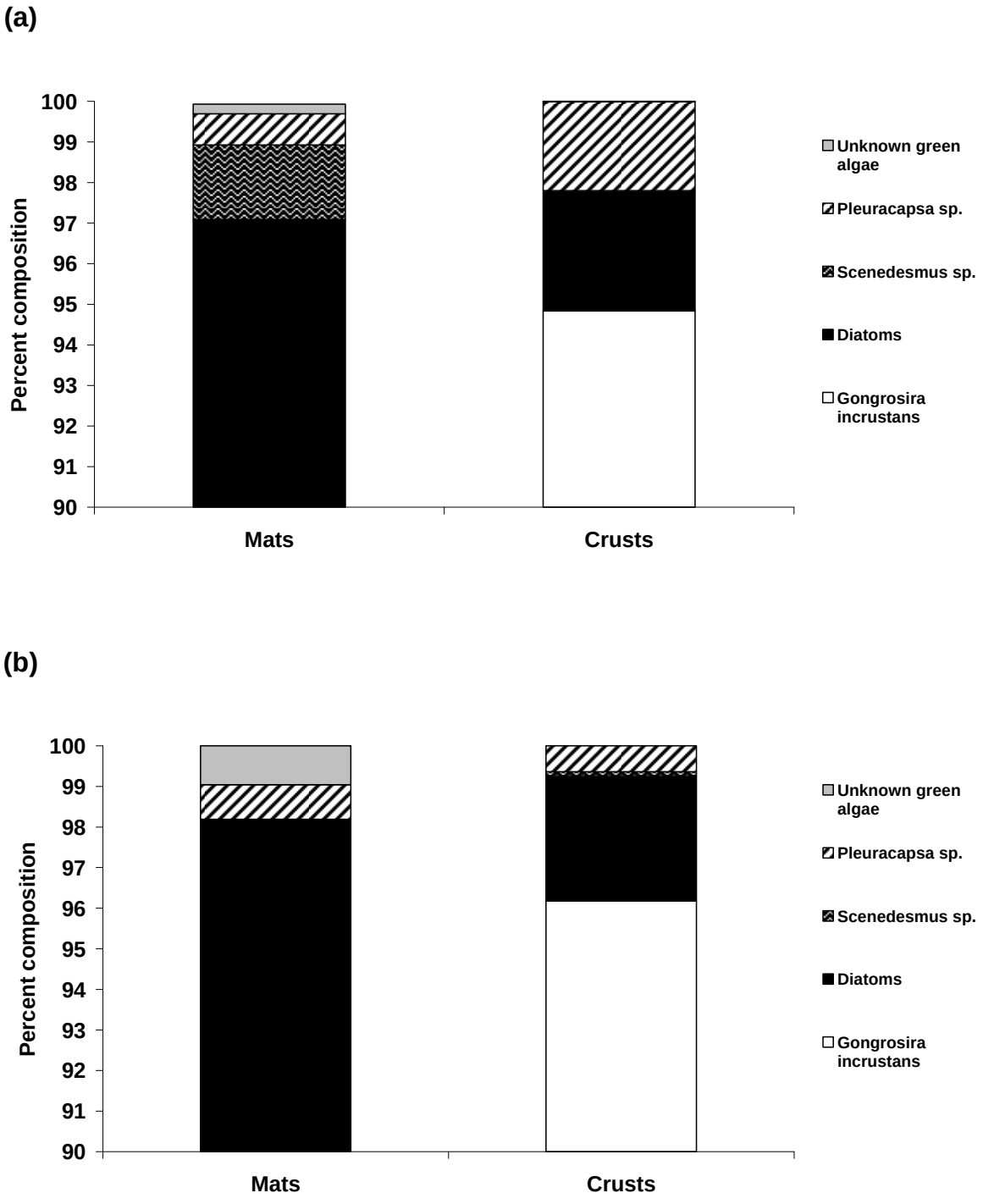
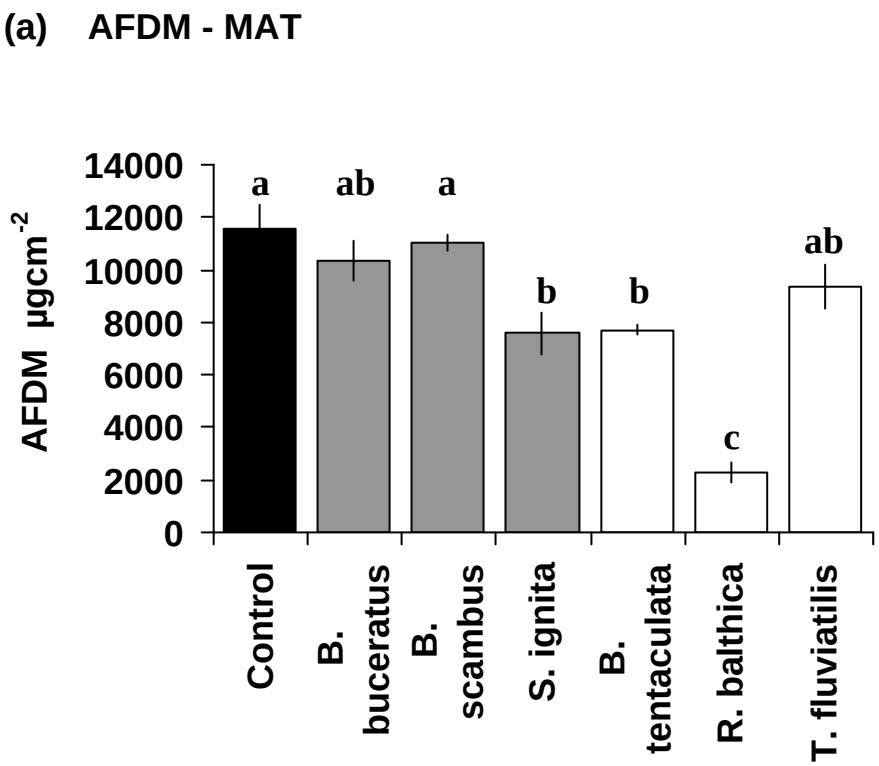


Figure 3. 4 Means + 1 SE algal biomass estimates of mat biofilms for grazer treatments and ungrazed control. a) AFDM and b) Chlorophyll *a*. Different letters represent significant differences between treatment means.



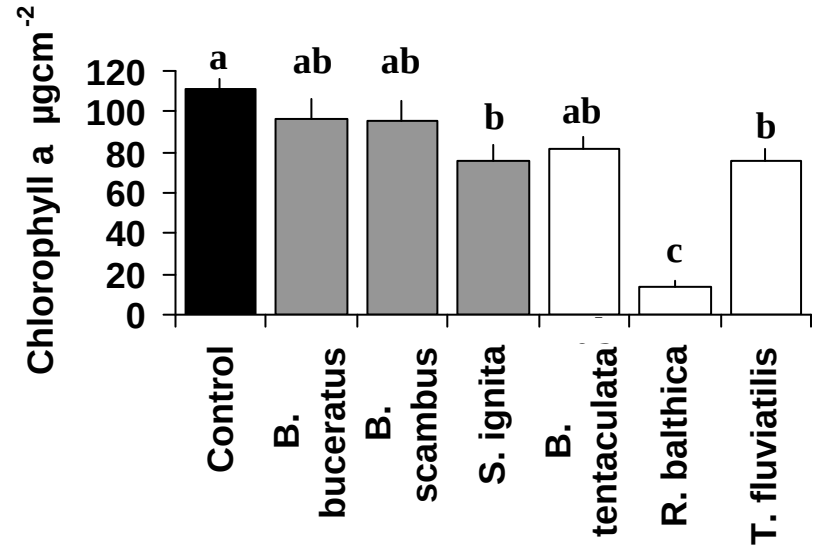
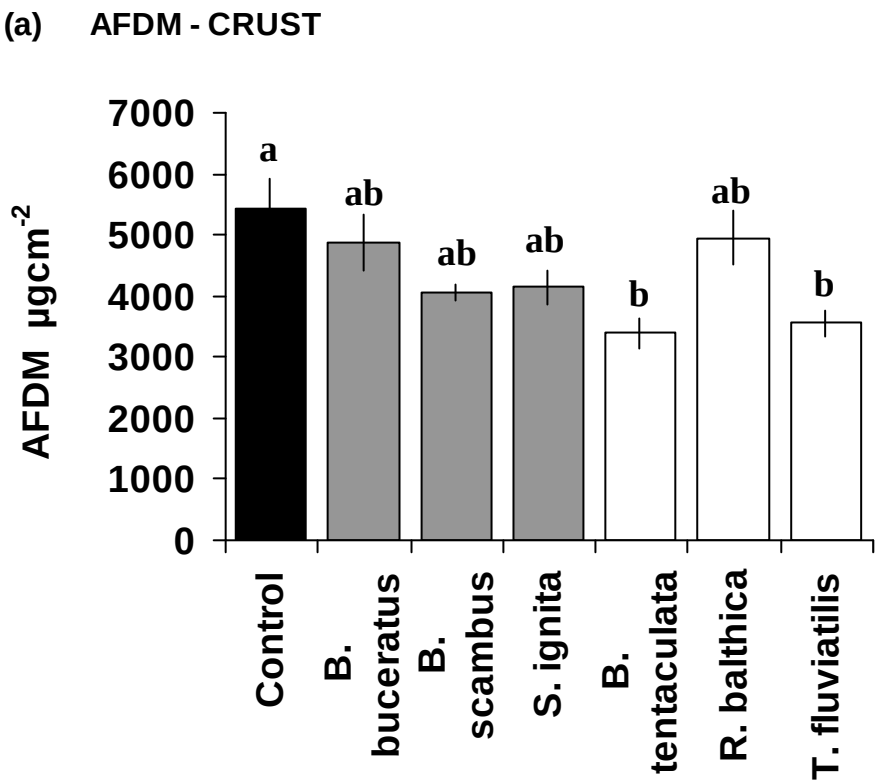


Figure 3. 5 Mean + 1 SE algal biomass estimates of crust biofilms for grazer treatments and ungrazed control. a) AFDM and b) Chlorophyll *a*. Different letters represent significant differences between treatment means.



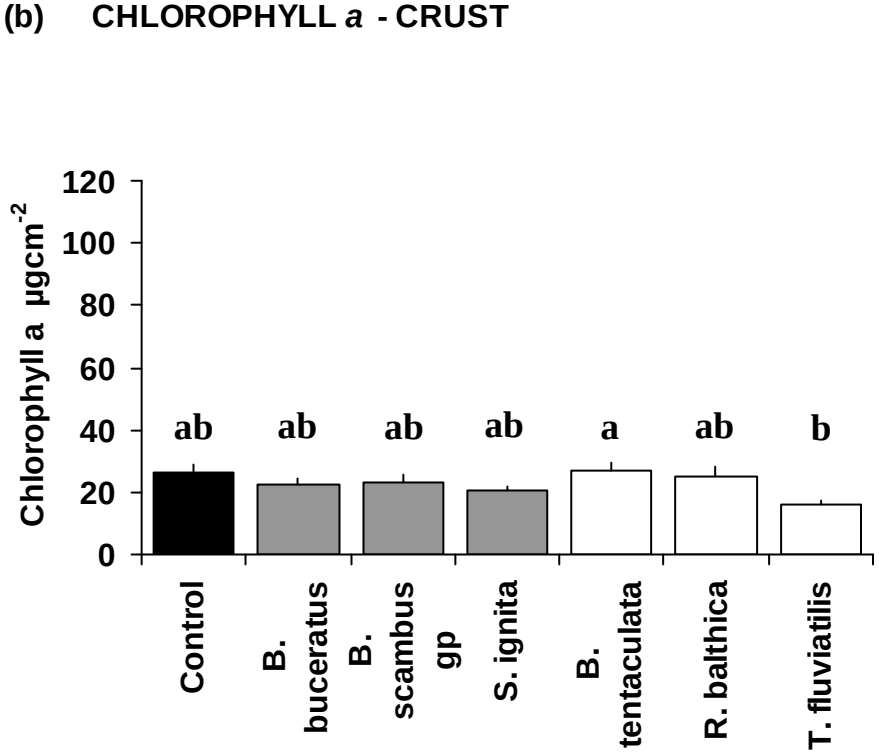


Figure 3. 6 Mean + /- 1 SE grazer per capita interaction strengths on algal biomass estimates a) AFDM and b) Chlorophyll *a*. are plotted. A Dynamic index of 0 indicates no interaction. Different letters represent significant differences between treatment means.

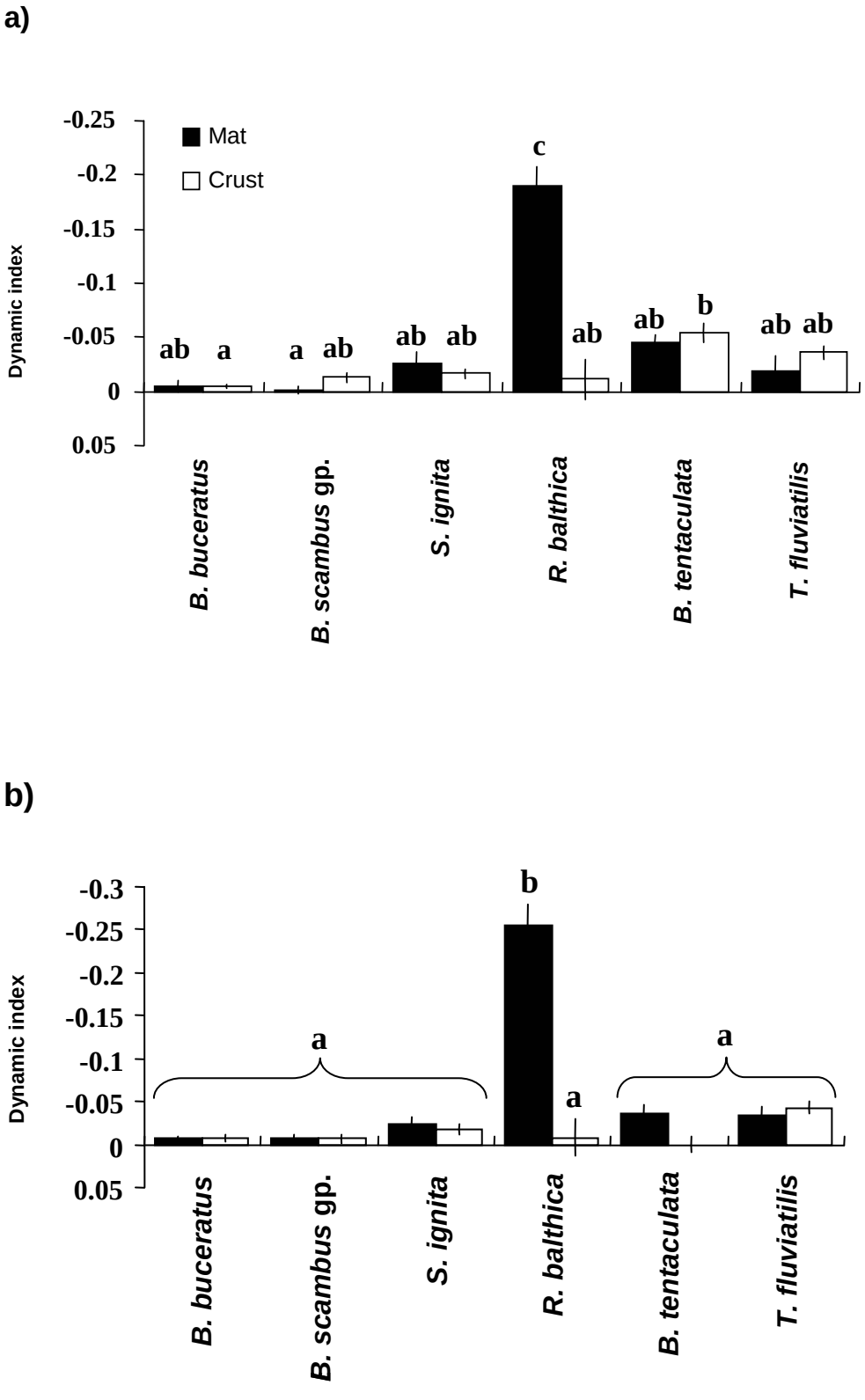


Figure 3. 7 Percentage frequency distribution of Dynamic Index values for grazer species on individual algal taxa. Black bars = Mats, White bars = Crusts.

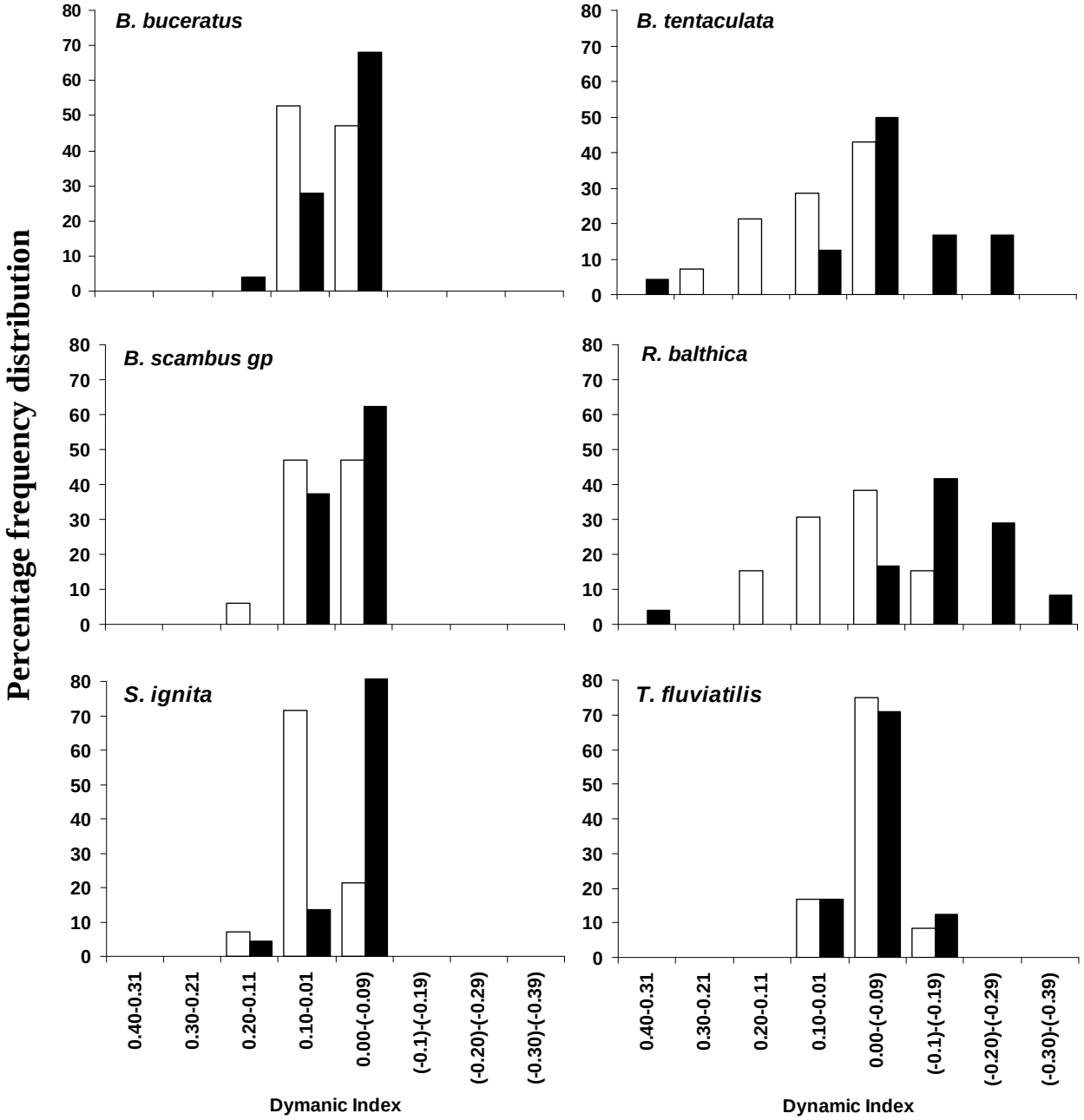
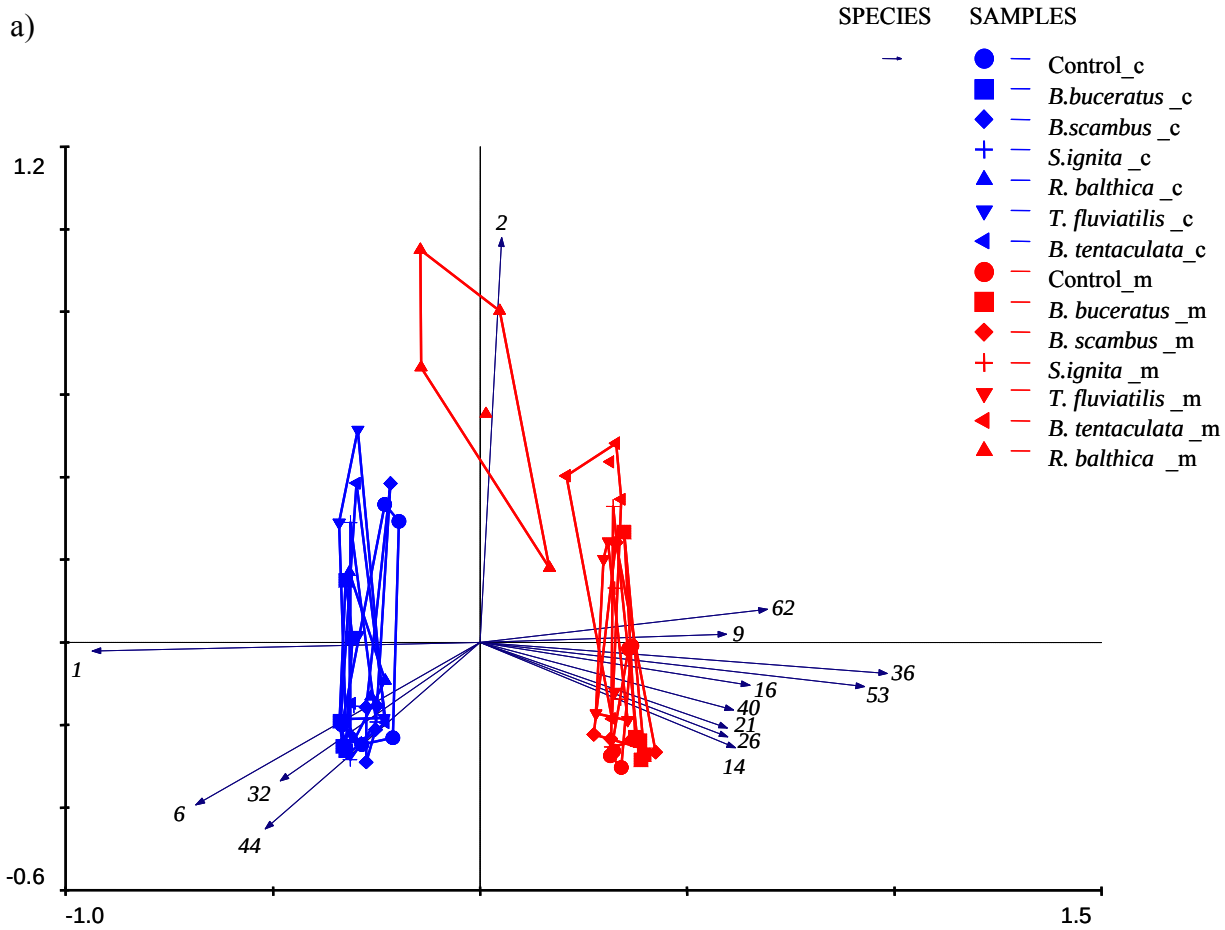


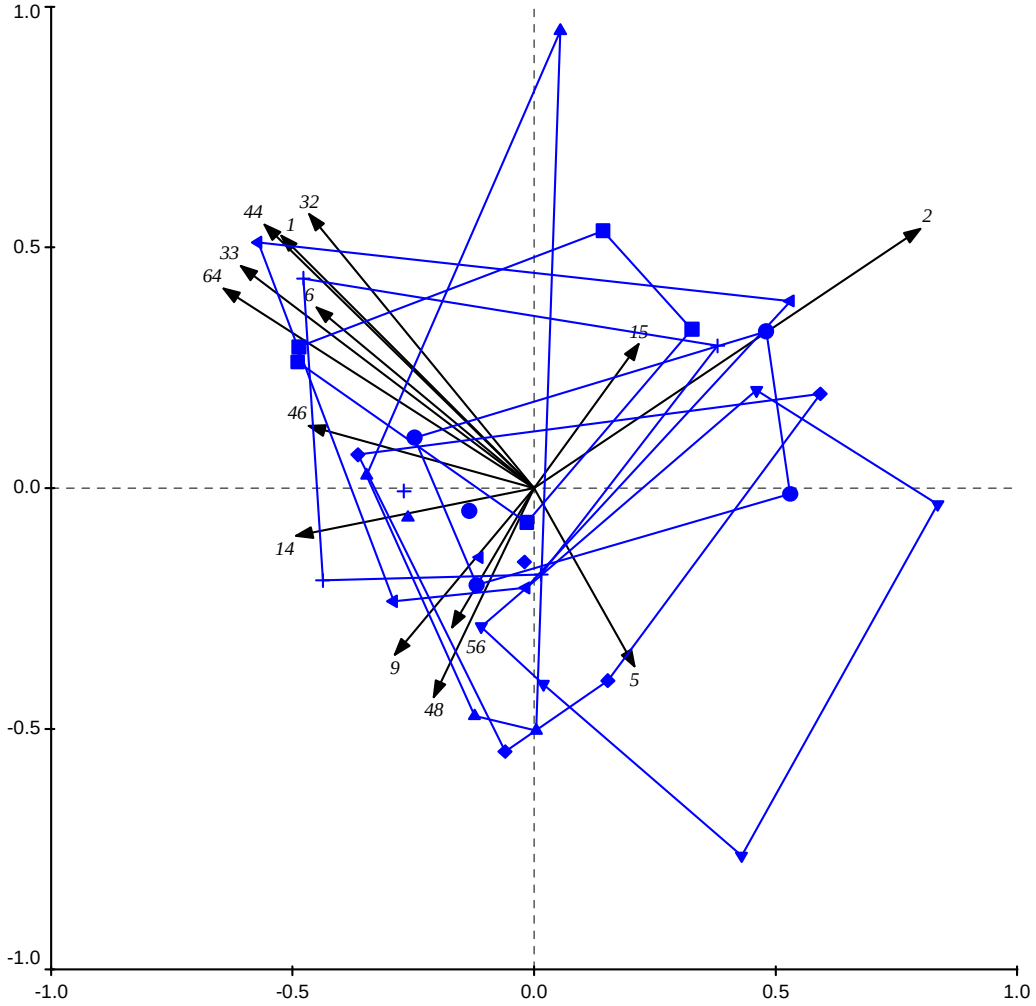
Figure 3. 8 Ordination plot of first two PCA axis using log x+1 species abundance data. a) Crusts and mats b) crusts only c) mats only. The legend is the same for all three graphs.

Symbols represent grazer treatment sample points. Mats in red and crusts in blue. Envelopes are drawn round sample points from the same treatments. Algal species are represented by vectors. 1, Gongrosira incrustans; 2, Pleurocapsa sp.; 4, Blue-green algae; 5, Green algae indet.; 6, Achnanthidium minutissimum; 8, Amphora ovalis; 9, Amphora pediculus; 14, Cocconeis placentula; 15, Cocconeis sp.; 16, Craticula halophila; 18, Cymatopleura solea; 21, Encyonema minuta; 26, Fragilaria sp. (GV); 29, Gomphonema angustatum; 32, Gomphonema parvulum; 33, Gomphonema sp. (GV); 36, Melosira varians; 40, Navicula cryptotenella; 44, Navicula menisculus; 46, Navicula tripunctata; 48, Nitzschia amphibia; 49, Nitzschia dissipata; 52, Nitzschia sinuata; 53, Nitzschia small < 50µm; 56, Planothidium gigantissimum; 60, Surirella brebissonis; 62, Small Navicula/Achnanthidium; 64, Indet. Diatom

a)



b)



c)

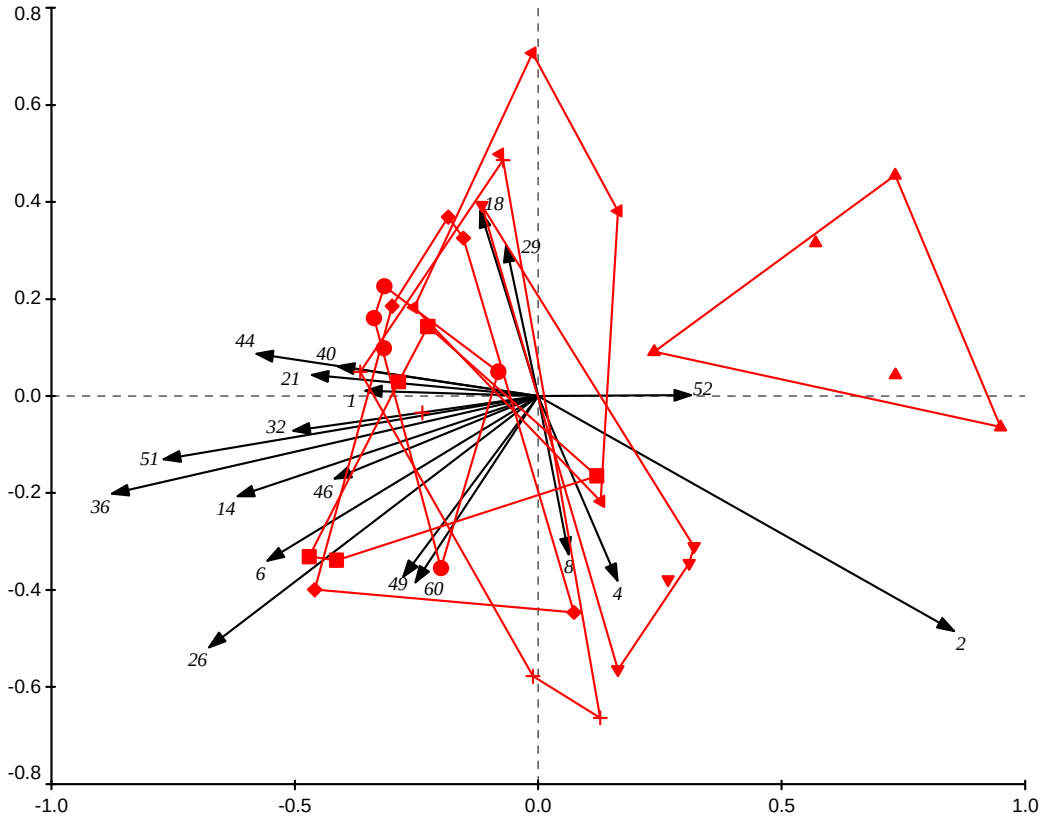
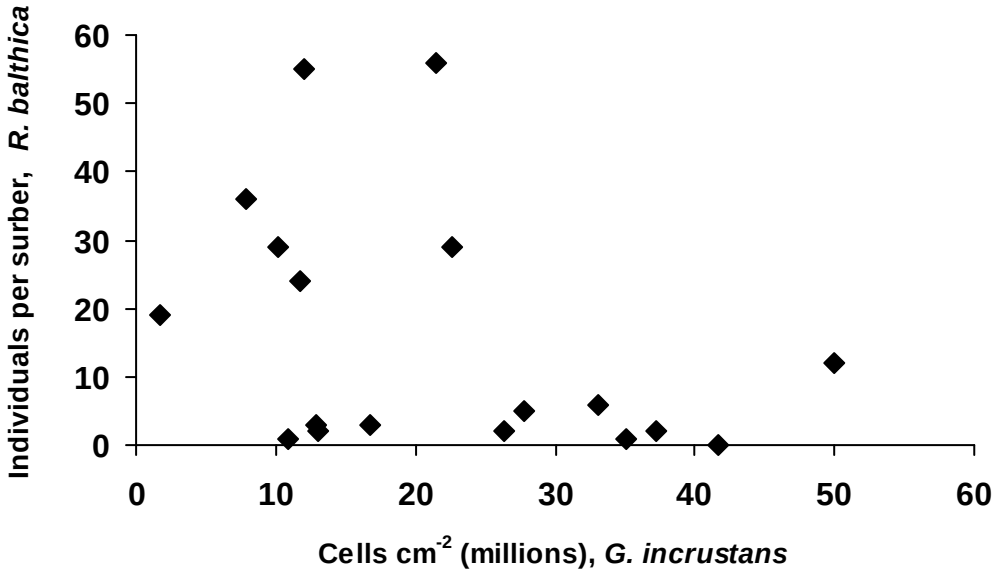

0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-0.5
0.0
0.5
1.0
18
29
44
40
21
1
52
32
51
36
14
46
6
49
60
8
4
2
26

Figure 3. 9 Relationship of *Radix balthica* density with *Gongrosira incrustans* density in the Mill stream during the experimental period. Spearman's rank correlation coefficient -0.458, $P = 0.065$



CHAPTER 4 - THE ROLE OF BODY SIZE IN ALGAL HERBIVORE INTERACTIONS

4.1 Summary

The relationship between herbivore body size and algal resource depression was investigated using short duration experimental manipulations. Three herbivore species (*Radix balthica*, *Serratella ignita* and *Agapetus* spp.) were grouped as small, medium and large bodied. Experiments were conducted in mesocosms located on the Mill Stream in Dorset. Body size was found to contribute to grazer impacts, although grazer identity was more important for identifying strong interactions. Snail and caddisfly grazers exerted stronger impacts as they increased in size, but mayfly larvae were poor at reducing algae at all body sizes.

4.2 Introduction

4.2.1 *Herbivore interaction strength and body size*

Benthic macro-invertebrate grazers are widely abundant, contributing to substantial amounts of total biomass in freshwater lotic systems (Steinman, 1996). Grazing is a key function for the assimilation of biomass produced by benthic autotrophs (Rosi-Marshall and Wallace, 2002). This is of particular importance in streams where primary production is the dominant basal resource and grazers represent the main channel of energy transfer (Lamberti, 1996). Therefore, identifying critical assimilation pathways from the base of the food web is important for interpreting processes at higher levels, and subsequently for the entire web (Begon et al., 1996). Quantifying per capita resource depression by grazers also expands our current knowledge of food-web structure and link-strength between resource and consumers (chapters 2 and 3). The strength of these interactions can have important consequences for the stability (Yodzis, 1981; de Ruiter et al., 1995; McCann et al., 1998; Neutel et al., 2002) and functioning (Hulot et al., 2000; Duffy, 2002; Paine, 2002) of ecological communities. However, deriving interaction strengths across entire ecological networks presents a complex and time consuming conundrum for ecologists to resolve. Consequently, the use of biological surrogates, which exploit easily measured parameters to predict interaction strength, has gained popularity in recent years (Berlow et al., 2004; Emmerson and Raffaellie, 2004). One such proxy is body size (Sala and Graham, 2002; Berlow et al., 2004). Patterns of Interaction strength and body size relationships between predators and their prey are increasingly evident from the scientific literature (Jonsson and Ebenman, 1998; Emmerson and

Raffaelli, 2004; Woodward et al., 2005). Therefore, body size could be a key factor for identifying and understanding grazer effects on biofilms in natural systems.

4.2.2 *Ontogenetic development, population structure and interaction strength*

Throughout an organism's ontogenetic development, increasing body size will influence individual traits such as; metabolic rate, consumption and food selection (Cohen et al., 1993). For example, metabolic requirements increase with size, and create higher consumptive demands for a large body size (Woodward et al., 2005). However, once large enough, consumers attain a size refugium from their predators. This further facilitates higher consumption by decreasing predator-induced impacts on foraging. (Werner and Gilliam, 1984 and Chase, 1999). However, ontogenetic shifts in vulnerability to predation have important implications for metrics based on body size (Chase, 1999), as these assume vulnerability to predation remains constant across body sizes (Woodward and Hildrew, 2002 and Werner and Peacor, 2002).

Conversely in early ontogenetic development, small grazers, gape limited by the size of their mouthparts fail to exploit large resources (Anderson et al., 1999) but may still consume an equivalent biomass of smaller resources (Cattaneo and Kalff, 1986). As body size increases (ontogenetic progression) the size range of prey items expands (Woodward and Hildrew, 2002), and thus the range of prey species consumed will increase (Begon et al., 1996). Research indicates that per capita interaction strengths increase as consumer body size increases (Warren and Lawton, 1987; Steinman, 1991; Jonsson and Ebenman, 1998; Munzo et al., 2000; Woodward et al., 2005). Furthermore, per capita interactions decrease as over crowding increases (Steinman, 1991 and Cattaneo and Mousseau 1995), thus suggesting strong interactions are likely

to occur in less crowded populations of large sized individuals. Consumer species with discreet lifecycles where overlap among cohorts is minimal, such as herbivorous insect larvae (*Serratella ignita* and *Agapetus* sp.), may experience temporal changes in population interaction strengths, while consumers with overlapping cohorts that have mixed size classes at anyone time, such as *Radix balthica* may see less temporal variation in population interaction strengths. However, competitive interactions are stronger among larger con-specifics (Steinman, 1991, Boaventura et al., 2003) leading to higher mortalities (Boaventura et al., 2003) which may then reduce impacts on producers. Consequently, the population interaction strengths of many small individuals may in fact be similar in magnitude to populations of fewer larger individuals (Cattaneo and Kalff, 1986, Chase, 2003).

4.2.3 *Body size relationships between consumers and their food resource*

Many studies focus on organisms from higher trophic levels where the body size of consumers and their prey are within a few orders of magnitude (e.g. Emmerson and Rafaelli, 2004). It remains to be tested extensively whether consumers which are several orders of magnitude larger than their prey exert similar patterns of interaction strength with body size e.g. invertebrate grazers and their algal resources. In typical predator-prey relationships, prey size increases with predator size, because the predator switches to larger prey with ontogeny (e.g. *Rhyacophila dorsalis*, a freshwater caddisfly larva, Elliott, 2006), or because preferred prey grow at similar rates to the predator (e.g. predatory stoneflies and their mayfly prey, Allan, 1982). However, most individual benthic microalgae are significantly smaller than grazing macro-invertebrates (Steinman, 1996, Brose et al., 2006) and the relative difference in

size increases as grazers grow while algae remain several orders of magnitude smaller. Consequently, as a grazer grows its ability to meet metabolic requirements through consuming larger prey types will reach a threshold at the maximum prey size (Sala and Graham, 2002). Beyond this point larger grazers will have to consume greater quantities of prey to continue to grow, and this may include a range of algal types and sizes (Tall et al., 2006b). However, due to the mode of attachment to the substrate, rather than intrinsic cell size alone, some algal species are more susceptible to grazing than others (Tuchman and Stevenson, 1991; Steinman, 1996). For example, some diatom species reach a refugium from grazing through the formation of filaments (Sumner and McIntire, 1982; Stevenson, 1997) composed of chains of cells linked together (*Melosira varians* and *Fragilaria* spp.). Thus, the impact grazers have on different components of the biofilm varies with their body size. Small grazers will remove smaller algal species closely attached to the substrate (adnate) as these are within the range of their mouthparts (Sumner and McIntire, 1982). As the grazer grows, its ability to traverse the vertical aspect of the biofilm will enable it to consume larger loosely attached and stalked algae (overstory species) in addition to adnate species (Tall et al., 2006a).

Typically grazer impacts are quantified using effects on algal biomass, with increases in the size of consumers leading to reductions of algal biomass (Steinman, 1996; Fagan and Hurd, 1994). Comparing resource depression across grazer types by using algal biomass provides a summary of how body size will affect algal productivity at a coarse level. However, comparisons of algal assemblage structure and the size of cells within that assemblage can elucidate the finer mechanisms involved (Tall et al., 2006b). Studying the impact of body size at these levels will provide a comprehensive picture of grazer-algae interactions, and a more mechanistic understanding of

interaction strength (Abrams, 2001; Woodward et al., 2005). Thus, to be able to predict interaction strength patterns, research is needed to elucidate how the size and identity of grazers interact with the size and identity of algae.

This experiment was designed to investigate body size – interaction strength relationships between macro-invertebrate grazers and algal biofilms. The effects of three morphologically distinct grazers (caddis flies, mayflies and snails) on algal size and abundance were tested across three size classes (small, medium and large). The relative importance of grazer size and identity was compared. Algal resource depression was expected to depend on 1) Grazer size: as grazers increased in size, or the grazer-algae size disparity increased, per capita interaction strength was expected to increase; 2) Grazer identity: caddisflies and snails were expected to exert stronger per capita interactions than mayflies.

4.3 Methods

4.3.1 *Study site / Experimental background*

An experiment was conducted in mesocosms (see chapter 2 for a full description) in June and July 2006 at the Freshwater Biological Association's fluvarium.

4.3.2 *Biofilm*

Algal biofilms were allowed to colonise unglazed ceramic tiles (2.5cm x 2.5 cm) in bank side experimental channels over a three month period, from June to July 2006. Biofilms were disturbed frequently during the colonisation period to prevent the dominance of *Gongrosira incrustans* on biofilms. Tiles were then selected at random for experimentation and transferred to the Fluvarium.

4.3.3 *Grazers*

Three grazer species were selected from the following groups; gastropod molluscs (*Radix balthica*), mayfly larvae (*Serratella ignita*) and cased Caddisfly larvae (*Agapetus* spp.). *Serratella ignita* and *R. balthica* were selected to build on previous data collected in chapters 2 and 3. *Agapetus* spp. was selected in order to assess the importance of a caddisfly larva, as a grazer, in comparison with snails and mayflies. These three species are amongst the most abundant herbivores in local chalk streams within the Frome-Piddle catchment, and therefore, represent the dominant components of the herbivore guild in these systems (Ladle and Bass, 1981; Armitage

et al., 2003; Davy-Bowker et al., 2006). *Serratella ignita* and *Radix balthica* were numerous in the Mill stream, while *Agapetus* ssp. were abundant in the nearby Bere Stream.

Invertebrates were collected from the Mill Stream (*Radix* and *Serratella*) and Bere Stream (*Agapetus*) using a standard kick net (300 μm mesh) in July 2006. Each species was sorted into three size classes; small medium and large. Grazers were measured in the field by placing individuals in a Petri dish of stream water over laminated graph paper. Individuals in each size class varied with each species of grazer and its availability. Consequently, small medium and large size classes were different across grazer species. Typically, snails were of larger size than larvae of both mayflies and caddisflies. Densities of grazers within each treatment were assigned based on body size, for example, as grazer body size increased individuals were added at lower densities (table 4.1).

4.3.4 *Experimental design*

Small, medium and large treatments, for each grazer and an ungrazed control, were replicated five times (five blocks) and randomly assigned to experimental mesocosms. The size disparities among species necessitated a factorial design (3 size classes x 3 species x 5 replicates) in which grazer body size was nested within grazer identity.

Replicates were assigned to five experimental blocks. Individual blocks were suspended in the fluvarium channel by a buoyant polystyrene (3 cm thick) frame (see chapter 2). Each chamber received one randomly assigned algae-coated tile transferred from the nearby channel prior to the introduction of the herbivores. The number of animals added to each chamber varied depending on the treatment

combination (table 4.1) The duration of the experiment was kept to a minimum (70 hours) to limit any cage effects that may influence the feeding of the animals. Mesocosms were checked for grazer mortalities on a daily basis and any dead animals were replaced with a specimen of similar size. At the end of the experimental period grazers and algae were processed as for previous chapters. Additionally to previous chapters, the mean length (μm) of each benthic algal species was estimated from measurement of 10-20 algal cells (depending on abundance), to the nearest 1.0 μm using a calibrated compound microscope (x 1000 magnification) and digital imaging software, and algal taxa were subsequently classified into size classes (table 5.2); and the dimensions of grazers were measured to the nearest 0.01 mm using a calibrated eyepiece reticule and dissecting microscope. Shell height was measured for *R. balthica* and body length / head capsule width were measured for *S. ignita* and *Agapetus* spp.

4.3.6 *Data analysis*

The size of the grazers was compared between size treatments within species using one way ANOVA ($\alpha = 0.05$). Differences in size between small, medium and large treatments were not compared across species due to their inherent difference in morphology.

Per capita interaction strengths were calculated for each grazer treatment using the Dynamic Index: $DI = Ln(N/D)/Yt$ where, N = normal (treatment), D = deleted (ungrazed control), Y = grazer abundance, t = time (days). Dynamic Index estimates were calculated at the algal community level for AFDM and Chlorophyll *a*. Data were normality distributed with homogeneous variances (Anderson-Darling and two-tailed

Levene tests respectively, $\alpha = 0.05$). A factorial ANOVA compared dynamic index values across the main factor, gazer identity ($\alpha = 0.05$). Body size (small, medium and large) was nested within the main factor as grazer lengths were not equal across grazer species (figure 5.1). Significant differences between grazers were assessed with Tukey's multiple comparisons. A nested design does not allow pair wise comparisons of the nested factor, in this case body size. Consequently, differences between size classes were analysed using single one-way ANOVAs for each grazer species ($\alpha = 0.05$).

Size class distributions for the controls and each treatment were plotted. Size classes were expressed as a proportion of the total algal population. *G. incrustans* was excluded from this analysis because it did not vary in size and skewed results towards size class category three which swamped the other size class data. This crust forming species is relatively difficult for consumers to remove, and is weakly affected by grazers (chapter 3). Therefore, its importance in assessing size frequency distributions is debatable and not investigated further here.

Algal community composition of treatments and controls was analysed using a principal components analysis (PCA). Log_{10} x +1 transformed algal abundance data was used in this analysis. Algal species were grouped by size class (table 4.2), represented as vectors on the ordination plot. The first two principal components were used in the ordination diagram, as they carried most of the variance.

Predator (grazer) / prey (algae) body size ratios were calculated for all grazers present in the treatments at the end of the experiment. Algal prey body size ranged from <5 μm to > 150 μm, with the majority of cells identified between 30 – 100 μm. Algal cell size was not manipulated in this experiment; however the range in algal cell size was an order of magnitude smaller than grazer body size. Therefore, a maximum algal

cell size of 100 μm was assumed to calculate predator / prey body size ratios (i.e. including all cells up to the maximum size of the common algae). A two-tailed Spearman rho correlation was used to assess the correlation between predator / prey body size ratios and the per capita interaction strength on algal AFDM and Chlorophyll *a*. A nonparametric correlation test was used because the body size ratio variable did not meet the assumptions of regression and there was no reason to assume a linear relationship.

4.4 Results

4.4.1 *Grazer body sizes*

Within species; small, medium and large body sizes were significantly different (figure 4.1), i.e. for each grazer species, individuals in the small treatment were smaller than those in medium and large, while medium individuals were smaller than large individuals. Body lengths within size classes increased across grazer treatments; from *Agapetus* spp. to *R. balthica*.

4.4.2 *Per capita interactions with AFDM and Chlorophyll a – Dynamic Index*

The nested ANOVA showed significant differences in interaction strength (dynamic indices for both AFDM and Chlorophyll *a*) across grazer identity (table 4.3). Tukey's pair wise comparisons showed that the snail *Radix balthica* had a significantly greater impact on both AFDM and Chlorophyll *a* compared to the mayfly *Serratella ignita* and the cased caddisfly, *Agapetus* spp., which were not different from each other (figure 4.2). Within grazer treatments, the effect of size on interaction strength was significant for both AFDM and Chlorophyll *a* (table 4.3) Per capita grazer size impacts (Dynamic index) for AFDM and Chlorophyll *a*, analysed separately using one-way ANOVA, showed a significant difference across body sizes within the *R. balthica* grazer treatment (AFDM: $F = 36.4$, $P < 0.001$; Chlorophyll *a*: $F = 40.9$, $P < 0.001$). All other size treatments showed no difference in the means (figure 4.3). The effect of size nested within grazer species was dominated by the stepped effect of *R. balthica*. Large snails had a greater impact than medium or small snails, which

showed a similar effect on AFDM. For Chlorophyll *a* large snails had a greater impact than medium snails, which had a greater impact than small snails (figure 4.3). *Agapetus* spp. showed a similar, but statistically non significant pattern with size. *Serratella ignita* showed no distinct pattern and large variances were characteristic of the data.

4.4.3 *Algal size class distributions*

Algal size class distributions produced bimodal plots with two peaks. The highest proportion of cells occurred in size class six (26 - 30 μm maximum cell length), dominated by the chain forming diatom *M. varians*. A second slightly smaller peak occurred at size class two (5 – 10 μm cell length) for grazer treatments and class three (11 – 15 μm cell length) for controls. (figure 4.4).

4.4.3.1 *Size treatments pooled* (figure 4.4a)

The *Agapetus* spp. treatment produced similar proportions of small cells (5 – 10 μm) and large cells (26 – 30 μm). In contrast, *S. ignita* and *R. balthica* treatments clearly lowered the proportion of small cells to large cells. However, all grazers reduced the proportion of larger cells and increased smaller cells compared to controls.

4.4.3.2 *Size differences within grazer treatments – Agapetus* (figure 4.4b); *S. ignita* (figure 4.4c); and *R. balthica* (figure 4.4d)

Agapetus spp. produced narrow peaks for small and large algal cells (class two and six respectively) regardless of grazer size, but the height of the peaks varied with

grazer size. The proportion of small algal cells decreased with grazer size, and conversely the proportion of large cells increased with grazer size.

For *Serratella ignita* the proportion of large cells barely decreased from controls for large individuals but decreased slightly more for medium and small individuals. The proportion of small algal cells was similar to controls for medium and large *S. ignita*. However, small grazers caused a shift towards a higher proportion of small algal cells compared to controls.

Small *R. balthica* depressed the proportion of small algal cells but had little impact on the proportion of large algal cells. Compared to controls medium-sized snails dramatically altered algal size-class proportions; reducing the numbers of the large algal cells and increasing the numbers of small cells. Large *R. balthica* produced an almost identical distribution to the control.

4.4.3.3 *Summary of distribution data*

Most grazers treatments, compared to controls, caused a shift in small algal cells from 11-15 μm to 5-10 μm. This was mainly due to an increase of the blue / green alga *Pleurocapsa* spp. (class 2) and a decrease in the basal adnate diatoms *Achnanthidium minutissimum* and *Amphora pediculus* (class three). The proportions of the dominant size classes varied with grazer size, especially for *Agapetus* spp. and *R. balthica* treatments. Small and medium grazer treatments varied substantially depending on the grazer identity, while large grazers and in particular *R. balthica* tracked control algal size class distributions more closely. Treatments also reduced the proportion of algal cells in size class seven (31 – 40 μm) compared to controls. This size class was dominated by the basal adnate diatom species *Cocconeis* spp.

4.4.4 *Ordination of treatments with algal size class distributions*

In the algal abundance data set (size class level), a high proportion of the variance, 59.2%, was explained by Principal Component 1, and a further 8.8% was explained by Principal Component 2, in total 68% of the variation. Overall, the ordination explained up to 81.4% of the variation within the algal abundance data, thus this model was a good fit to the community data (table 4.4). *Radix balthica* (large) treatments were most similar to controls, which were characterised by the dominance of size classes three, six and seven. The remaining *R. balthica* treatments (small and medium) were spread throughout the ordination, and could not be clearly separated from *Agapetus* spp. and *S. ignita* treatments. *Agapetus* spp. and *S. ignita* treatments overlapped, but showed the strongest shifts from control treatments and were clearly separate from *R. balthica* large treatments on the primary axis (figure 4.6).

Radix balthica and control treatments were best described by algal size classes three (11 - 20 µm), six (26 - 30 µm) and seven (31 - 40 µm). Size class three was dominated by basal adnate diatoms; *A. minutissimum* and *A. pediculus*. Classes six and seven were dominated by the chain forming diatom *M. varians*, *Fragilaria* spp. (class six), *Cocconeis* spp. and small *Nitzschia* spp. (< 50 µm, class seven). *Agapetus* spp. and *S. ignita* treatments were characterised by algal size class two (5 - 10 µm), with the colony forming *Pleurocapsa* spp. the sole species assigned to this size class.

4.4.5 *Predator / prey body size ratio relationship with per capita interaction strength*

Grazer / algal body size ratios were significantly correlated with per capita impacts on algal AFDM and Chlorophyll *a* (figure 4.7) (AFDM and Body size ratio correlation

coefficient (r_s) = 0.52, P = < 0.001; Chlorophyll *a* correlation coefficient (r_s) = 0.43, P = 0.003). Both AFDM and Chlorophyll *a* were positively correlated with body size ratios, with AFDM showing a slightly stronger relationship than Chlorophyll *a*. Therefore, as the size ratio increases (i.e. grazer size decreases and is closer to algal cell lengths) per capita interaction strength decreases, but non-linearly.

4.5 Discussion

4.5.1 *Per capita impacts on algal biomass: identity and body size*

Herbivore-algae interaction strength varied with grazer identity as snails had greater impacts than mayflies and caddis flies. *Radix balthica* significantly depressed algal resources compared to *S. ignita* and *Agapetus* spp. for both AFDM and Chlorophyll *a*. *Radix balthica* is an area extensive bulldozer (Wilson et al., 1999; Chase et al., 2001) proficient at cropping the majority of stalked and loosely attached algal forms using its specialised radula, furthermore loosely attached material is easily sloughed as it powers through the biofilm (Chapter 3; Steinman, 1996). By comparison *S. ignita* barely affected algal biomass. Fuller and Desmond (1997) found that as mayflies grew their use of algal resources increased, with weight gain of later instars related to algal consumption. However, highly variable impacts were observed across mayfly size classes, suggesting size may not be an overriding determinant of mayfly impacts in this study. *Serratella ignita* has delicate brush-like mandibles and a crawling / swimming mode of locomotion which restricts its consumption to browsing erect, stalked and filamentous forms of algae (Chapter 3; Steinman, 1996; Poff et al., 2003; McNeely et al., 2007). Additionally, *S. ignita* is less likely to dislodged loosely attached algae as it darts around the substrate (Cattaneo and Mousseau, 1995; Steinman, 1996). The cased caddisfly *Agapetus* spp. was also weak at reducing algal biomass compared to *R. balthica*. Poff et al., (2003) found the grazing capacity of the cased caddisfly *Glossosoma verdona* decreased at lower flow velocities, with their preferred velocity > 25cm^{-s} (Hart, 1987). *Agapetus* spp. and *Glossosoma* spp. are taxonomically similar, thus it is reasonable to assume *Agapetus* spp. may also respond

poorly to the restricted flow in experimental mesocosms. Hart (1987) found *Glossosoma* larvae impacts on algal biomass were greatest at double natural densities, suggesting effects may be more detectable as densities exceeding that of natural populations. Furthermore, this grazer was the smallest of the three species tested in each size group, yet its effect was similar in magnitude to *S. ignita* consequently, removal of algal biomass per unit length of grazer was greater for *Agapetus* spp.

Radix balthica and *Agapetus* spp. produced a gradient of per capita interaction strength from small to medium to large individuals; but this gradient was only significant for *R. balthica*. Although individuals within size treatments for each species had significantly distinct size ranges, the difference in size between small, medium and large was less pronounced for the *Agapetus* spp. treatment than the *R. balthica* treatment. *Agapetus* spp. vary little in length from first to final instars (0.7 – 5 mm), whereas the shell height of *R. balthica* ranged from 3 – 13 mm. Increased resource consumption from small to large individuals reflects associated increases in metabolic requirements during the growth of the grazer (Steinman, 1991; Cattaneo and Mousseau, 1995). Size impacts of *Agapetus* spp. were detectable but with such a narrow range of body size were not statistically significant. Unlike *R. balthica* and *Agapetus* spp., *S. ignita* showed no pattern in interaction strength across body size treatments, principally due to large variances between replicates. Overall, interaction strength was therefore dependant on both identity and size of grazers, but the effect of size varied with grazer identity.

During the manipulation of experimental animals exposure to physical stress is unavoidable. However, each species reacts to, and tolerates this disruption differently. For example, delicate mayflies and small caddis are more awkward for the experimenter to handle than *Radix balthica*, which is far easier to manipulate

manually (personal observation). Furthermore, snail mortalities during the experimentation process did not occur, unlike for mayflies and caddis larvae which required some additions to replace dead and emerged individuals. Furthermore, smaller insect larvae may suffer more from physical manipulation than their larger counterparts (personal observation).

In summary, the effects of physical manipulation, mouthparts and foraging performance appear to reduce insect larvae effects on algal biomass. This has important implications for real ecosystems, in that experimental methods that involve broad measures of effect (i.e. algal biomass) may not yield detectable effects from weakly interacting species. This highlights the importance of measuring several response variables to quantify effect (Feminella and Hawkins, 1995), such as algal production, and community structure. Furthermore, detection of effects may be compounded by susceptibility to experimentation procedures, which was clearly evident for the insect larvae used in this chapter. Exposure to stress during experimentation is not easily avoided. Consequently, it may be appropriate to factor some measure of stress into interaction strength calculations.

4.5.2 *Grazer affects on the algal community structure*

Distribution of algal cell size classes were clearly affected by *R. balthica* treatments. Small individuals reduced numbers of small algal cells but didn't affect large cells; because limitations of gape and/or mouthparts determine the resources a grazer can consume (Begon et al., 1996; Steinman, 1996). In contrast, medium *R. balthica* were less restricted by gape and mouthpart size and removed greater proportions of large algal cells. Small cells, predominantly *Pleurocapsa* spp., increased in proportion in

the medium *R. balthica* treatment. This was coupled with reductions in *M. varians* (occupying upper algal layers) and adnate diatoms in the understory, such as *A. minutissimum*, *A. pediculus* and *Cocconeis* spp. Loss of the overstory layer promotes growth and turn-over rates of species lower in the biofilm, by increasing light and nutrient availability from the water column (Hill and Knight, 1988; Bronmark et al., 1992; Underwood et al., 1992; Biggs and Lowe, 1994; Diaz Villanueva and Modenutti, 2004; Liess and Kahlert, 2007). With an associated decrease in competitors (adnate species) at the basal level, species like *Pleurocapsa* spp. can rapidly accumulate because they are released from resource competition (Peterson, 1996; Steinman, 1996). Increased turnover of *Pleurocapsa* spp. combined with weak grazer depression, possibly a function of selective grazing to avoid less nutritious resources (Brown et al., 1997), could explain the increase in this species in the medium *R. balthica* treatment. Large *R. balthica* reduced algal biomass significantly, but maintained an almost identical algal size class distribution compared to ungrazed controls. This could be due to a lack of food selectivity due to their large size and gape (Brendelberger, 1997). However, it is also possible the density of large *R. balthica* is important in explaining this result. In the 'large' treatments, single individuals of *R. balthica* were assigned to mesocosms to attain comparable grazer biomass across body size treatments. The grazing behaviour of single animals may deviate from treatments with higher densities because of reduced intraspecific competition (Begon et al., 1996), particularly if resources are abundant. Unrestricted by behavioural responses to competition, non-selective grazing by single large *R. balthica* could result in proportional resource depression where cell size class distributions remain unaffected. This is supported further by the lack of *Pleurocapsa* spp. (size class two). An intact biofilm, except in the immediate vicinity of grazing *R.*

balthica, would retain undisturbed adnate and overstory layers. Therefore, *Pleurocapsa* spp. would not be released from resource competition and increase in the same way compared to other treatments. Thus, large *R. balthica* treatments produced algal communities that were the most similar to controls, compared to the other treatments. Perhaps in the absence of competition, the wandering nature of *R. balthica* is less significant, thus allowing it to assume a digger role as opposed to a grazing role (see Wilson et al., 1999 and Chase et al., 2001).

Serratella ignita and *Agapetus* spp., although weak at reducing algal biomass compared to snails (see previous section), appeared to shift the size distribution of algal cells and more specifically the assemblage composition towards the smaller blue/green *Pleurocapsa* spp., regardless of grazer size, unlike snail effects which were strongly size dependent (figure 4.5). The community ordination also indicated that insect larvae created a more obvious shift in algal composition, which appeared to be driven by the reduction of biofilm overstory species (*M. varians*, *Fragilaria* spp.). Although overstory species form large filaments and chains the constituent cells are small and perhaps more easily picked off by all sizes of insect mandibles. Insect impacts occurring on upper algal layers are well documented (Hill and Knight, 1987, 1988; Feminella and Hawkins, 1995). Whereas snail radulae may remove algal cells as they occur in situ including whole filaments (see Jacoby 1985, where whole algal filaments were observed in *T. fluviatilis* guts). In which case algal size refugia may be important for snail removal, where large *Melosira* filaments are potentially less vulnerable to small snail grazers. Thus, algal size and identity also plays a role in algal-grazer interactions in accordance with other studies (e.g. Tall et al., 2006b).

The variable effects of grazer size and species identity demonstrated here and in other studies (Steinman et al., 1987; McCormick, 1994) demonstrate the importance of

measuring community level responses to grazing when quantifying interaction strengths.

4.5.3 *Body size ratios and per capita interaction strength*

The predator / prey body size ratio was a moderate predictor of per capita interaction strength in this experiment. Nonetheless, the relationship between the two suggested, that interaction strength increases with the disparity between predator and prey body size (see Woodward et al., 2005; Brose et al., 2006). However this data was calculated from the maximum size within the range of algal cells available, and thus resulted in ratios biased towards larger prey items. A finer scale approach might yield a stronger correlation between predator / prey size and per capita interaction strength. Gut content analysis would provide an accurate measure of predator / prey body size ratios. Using gut analysis, algal cell size-class distributions can be attained for individual grazers based on actual consumption rather than on the cells remaining in treatment biofilms (trophic versus non-trophic impacts on primary producers). However, this might create a bias towards less digestible algal species that are more easily recognised in the gut, e.g. the siliceous frustules of diatom species (Peterson, 1987; Foale and Day, 1992). In this experiment the non-diatom *Pleurocapsa* spp. (blue / green alga) increased under grazing, which shifted algal cell size class distributions. This alga is less likely to survive intact in a grazers gut. Therefore, data could be lost if gut analysis alone was used to estimate body size ratios (Peterson, 1987; Tall et al., 2006a).

The ability to quantify species interaction strengths from predator and prey body sizes provides ecologist with a tool that can estimate the interaction of species in highly

complex webs relatively simply (Sala and Graham, 2002; Berlow et al., 2004; Woodward et al., 2005). Body size as an interaction strength proxy has been found to work relatively well for larger organisms in aquatic food webs and in particular engulfing predators (Emmerson and Rafaelli, 2004). Unfortunately, relatively little information on small organisms, such as micro algae, and their much larger predators (macro invertebrate grazers) is available (Sala and Graham, 2002). The present study suggests that at smaller scales a relationship between body size and interaction strength exists, and warrants further investigation if freshwater food webs containing benthic algae are to be quantified accurately.

4.5.4 *Body size comparisons across grazing species*

Differences in grazer life-history strategies and maximum size attainment (e.g. insects are restricted by exoskeleton and maximum instar size compared to snail growth which is only restricted by nutrient availability), led to comparisons in this study of grazers with different lengths within the large, medium and small size classes. However, small individuals of one species may be less likely to experience interspecific competition from small individuals of another species when their sizes are unequal because niche overlap is reduced (May, 1974 and Werner and Gilliam, 1984), and so on for medium and large individuals. Consequently, interspecific competition between grazer species that differ morphologically, such as mayflies, snails and cased caddis flies may not be as important as intraspecific competition within a cohort where body size varies little as individuals develop and grow.

4.5.5 *Further investigation*

The impact of a single large *R. balthica* on biomass depression was clearly evident. However, in this experiment *R. balthica* were released from the ecological processes that usually constrain food acquisition and consumption, such as predation risk (Holt, 1977; Gurevitch et al., 2000), and competition with other grazers (McAuliffe, 1984). Therefore, it is important to investigate the influence of competition on grazer performance, to fully understand how interaction strength is distributed in complex natural systems. This is investigated in chapter 5.

4.6 References

Abrams, P. A. (2001) Describing and quantifying interspecific interactions: a commentary on recent approaches. **Oikos**, 94: 209-218.

Allan, J. D. (1982) Feeding habits and prey consumption of three setipalpian stoneflies (Plecoptera) in a mountain stream. **Ecology**, 63: 26-34.

Anderson, E. L., Welch, J. M., Jacoby, J. M., et al. (1999) Periphyton removal related to phosphorus and grazer biomass level. **Freshwater Biology**, 41: 633-651.

Armitage, P. D., Szoszkiewicz, K., Blackburn, J. H., et al. (2003) Ditch communities: a major contributor to floodplain biodiversity. **Aquatic Conservation: Marine and Freshwater Ecosystems**, 13(165): 185.

Begon, M., Harper, J. L. and Townsend, C.R. (1996) **Ecology: individuals, populations and communities**. 3rd edition. London, Wiley Blackwell. 1068 pp.

Berlow, E. L., Neutel, A. M., Cohen, J. E., et al. (2004) Interaction strengths in food webs: issues and opportunities. **Journal of Animal Ecology**, 73(3): 585-598.

Biggs, B. J. F. and Lowe, R. L. (1994) Responses of two trophic levels to patch enrichment along a New Zealand stream continuum. **New Zealand Journal of Marine and Freshwater research**, 28: 119-134.

Boaventura, D., Cancela De Fonseca, L. and Hawkins, S. J. (2003) Size matters: competition within populations of the limpet *Patella depressa*. **Journal of Animal Ecology**, 72: 435-446.

Brendelberger, H. (1997) Contrasting feeding strategies of two freshwater gastropods, Radix peregra (Lymnaeidae) and Bithynia tentaculata (Bithyniidae). **Archiv Fur Hydrobiologie**, 140(1): 1-21.

Bronmark, C., Klosiewski, S. P. and Stein, R. A. (1992) Indirect effects of predation in a freshwater benthic food chain. **Ecology**, 73(5): 1662-1674.

Brose, U., Jonsson, T., Berlow, E. L., et al. (2006) Consumer-resource body-size relationships in natural food webs. **Ecology**, 87(10): 2411-2417.

Brown, M. R., Jeffrey, S. W., Volkman, J. K., et al. (1997) Nutritional properties of microalgae for mariculture. **Aquaculture**, 151: 315-331.

Cattaneo, A. and Kalff, J. (1986) The effect of grazer size manipulation on periphyton communities. **Oecologia**, 69: 612-617.

Cattaneo, A. and Mousseau, B. (1995) Empirical analysis of the removal rate of periphyton by grazers. **Oecologia**, 103: 249-254.

Chase, J. M. (1999) Food web effects of prey size refugia: variable interactions and alternative state equilibria. **The American Naturalist**, 154(5): 559-570.

Chase, J. M. (2003) Strong and weak trophic cascades along a productivity gradient. **Oikos**, 101: 187-195.

Chase, J. M., Wilson, W. G. and Richards, S. A. (2001) Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. **Ecology Letters**, 4: 304-312.

Cohen, J. E., Pimm, S. L., Yodzis, P., et al. (1993) Body sizes of animal predators and animal prey in food webs. **Journal of Animal Ecology**, 62: 67-78.

Davy-Bowker, J., Sweeting, W., Wright, N., et al. (2006) The distribution of benthic and hyporheic macroinvertebrates from the heads and tails of riffles. **Hydrobiologia**, 563: 109-123.

De Ruiter, P. C., Neutel, A. M. and Moore, J. C. (1995) Energetics, patterns of interaction strength, and stability in real ecosystems. **Science**, 269: 1257-1260.

Diaz Villanueva, V. and Modenutti, B. (2004) Experimental analysis of grazing by the mayfly Meridialaris chiloeensis on different successional stages of stream periphyton. **International Review of Hydrobiology**, 89(3): 263-277.

Duffy, J. E. (2002) Biodiversity and ecosystem function: the consumer connection. **Oikos**, 99: 201-219.

Elliott, J. M. (2006) prey switching in *Rhyacophila dorslis* (Trichoptera) alters with larval instar. **Freshwater Biology**, 51: 913-924.

Emmerson, M. C. and Raffaelli, D. (2004) Predator-prey body size, interaction strength and the stability of a real food web. **Journal of Animal Ecology**, 73: 399-409.

Fagan, W. F. and Hurd, L. E. (1994) Hatch density variation of a generalist arthropod predator - population consequences and community impact. **Ecplogy**, 75: 2022-2032.

Feminella, J. W. and Hawkins, C. P. (1995) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. **Journal of the North American Benthological Society**, 14(4): 465-509.

Foale, S. and Day, R. (1992) Recognizability Of Algae Ingested By Abalone. **Australian Journal Of Marine And Freshwater Research**, 43(6): 1331-1338.

Fuller, R. L. and Desmond, C. (1997) Influence of food type on the growth of early and late instars of three mayfly (Ephemeroptera) species. **Archiv Fur Hydrobiologie**, 141(1): 1-11.

Gurevitch, J., Morrison, J. A. and Hedges, L. V. (2000) The interaction between competition and predation: a meta-analysis of field experiments. **American Naturalist**, 155(4): 435-453.

Hart, D. D. (1987) Experimental studies of exploitative competition in a grazing stream insect. **Oecologia**, 73: 41-47.

Hill, W. (1996) "Effects of light". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. **Chapter 7:** 184-218.

Hill, W. R. and Knight, A. W. (1988) Concurrent Grazing Effects of 2 Stream Insects on Periphyton. **Limnology and Oceanography**, 33(1): 15-26.

Holt, R. D. (1977). Predation, apparent competition and the structure of prey communities. Theoretical Population Biology. **12:** 495-520.

Hulot, F. D., Lacroix, G., Lescher-Moutoue, F., et al. (2000) Functional diversity governs ecosystem response to nutrient enrichment. **Nature**, 405: 340-344.

Jacoby, J. M. (1985) Grazing effects on periphyton by *Theodoxus fluviatilis* (Gastropoda) in a lowland stream. **Journal of Freshwater Ecology**, 3(2): 265-274.

Jonsson, T. and Ebenman, B. (1998) Effects of predator-prey body size ratios on the stability of food chains. **Journal of Theoretical Biology**, 193(3): 407-417.

Ladle, M. and Bass, J. A. B. (1981) The ecology of a small chalk stream and its response to drying during drought conditions. **Archiv fur Hydrobiologie**, 90: 448-466.

Lamberti, G. A. (1996) "The role of periphyton in benthic food webs". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 533-573.

Liess, A. and Kahlert, M. (2007) Gastropod grazers and nutrients, but not light, interact in determining periphytic algal diversity. **Oecologia**, 152(1): 101-111.

May, R. M. (1974) On the theory of niche overlap. **Theoretical Population Biology**, 5: 297-332.

McAuliffe, J. R. (1984) Resource depression by a stream herbivore - Effects on distributions and abundances of other grazers. **Oikos**, 42(3): 327-333.

McCann, K., Hastings, A. and Huxel, G. R. (1998) Weak trophic interactions and the balance of nature. **Nature**, 395: 794-798.

McCormick, P. V. (1994) Evaluating the Multiple Mechanisms Underlying Herbivore Algal Interactions in Streams. **Hydrobiologia**, 291(1): 47-59.

McNeely, C., Finlay, J. C. and Power, M. E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. **Ecology**, 88(2): 391-401.

Munoz, I., Real, M., Guasch, H., et al. (2000) Resource limitation by freshwater snail (Stagnicola vulnerata) grazing pressure: an experimental study. **Archiv Fur Hydrobiologie**, 148(4): 517-532.

Neutel, A. M., Heesterbeek, J. A. P. and De Ruiter, P. C. (2002) Stability in real food webs: weak links in long loops. **Science**, 296: 1120-1123.

Paine, R. T. (2002) Trophic control of production in a rocky intertidal community. **Science**, 296: 736-739.

Peterson, C. G. (1987) Gut Passage And Insect Grazer Selectivity Of Lotic Diatoms. **Freshwater Biology**, 18(3): 455-460.

Peterson, C. G. (1996) "Response of algae to natural disturbance". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 375-402.

Poff, N. L., Wellnitz, T. and Monroe, J. B. (2003) Redundancy among three herbivorous insects across an experimental current velocity gradient. **Oecologia**, 134(2): 262-269.

Rosi-Marshall, E. J. and Wallace, J. B. (2002) Invertebrate food webs along a stream resource gradient. **Freshwater Biology**, 47(1): 129-141.

Sala, E. and Graham, M. H. (2002) Community-wide distribution of predator-prey interaction strength in kelp forests. **Proceedings of the National Academy of Sciences of the United States of America**, 99(6): 3678-3683.

Steinman, A. D. (1991) Effects of herbivore size and hunger level on periphyton communities. **Journal of phycology**, 27: 54-59.

Steinman, A. D. (1996) "Effects of grazers on freshwater benthic algae". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** Academic Press. pp. 341-366.

Steinman, A. D., McIntire, C. D., Gregory, S. V., et al. (1987) Effects of herbivory type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. **Journal of the North American Benthological Society**, 6(3): 175-188.

Stevenson, R. J. (1997) Resource thresholds and stream ecosystem sustainability. **Journal of the North American Benthological Society**, 16(2): 410-424.

Sumner, W. T. and McIntire, C. D. (1982) Grazer-periphyton interactions in laboratory streams. **Archiv Fur Hydrobiologie**, 93(2): 135-157.

Tall, L., Cattaneo, A., Cloutier, L., et al. (2006a) Resource partitioning in a grazer guild feeding on a multilayer diatom mat. **Journal Of The North American Benthological Society**, 25(4): 800-810.

Tall, L., Cloutier, L. and Cattaneo, A. (2006b) Grazer-diatom size relationships in an epiphytic community. **Limnology And Oceanography**, 51(2): 1211-1216.

Tuchman, N. C. and Stevenson, R. J. (1991) Effects of Selective Grazing by Snails on Benthic Algal Succession. **Journal of the North American Benthological Society**, 10(4): 430-443.

Underwood, G. J. C., Thomas, J. D. and Baker, J. H. (1992) An experimental investigation of interactions in snail-macrophyte-epiphyte systems. **Oecologia**, 91: 587-595.

Warren, P. H. and Lawton, J. H. (1987) Invertebrate predator prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? **Oecologia**, 74: 231-235.

Werner, E. E. and Gilliam, J. F. (1984) The ontogenetic niche and species interactions in size structured populations. **Annual Review of Ecology and Systematics**, 15: 393-425.

Werner, E. E. and Peacor, S. D. (2003) A review of trait-mediated indirect interactions in ecological communities. **Ecology**, 84(5): 1083-1100.

Wilson, W. G., Osenberg, C. W., Schmitt, R. J., et al. (1999) Complementary foraging behaviours allow coexistence of two consumers. **Ecology**, 80(7): 2358-2372.

Woodward, G., Ebenman, B., Emmerson, M. C., et al. (2005) Body size in ecological networks. **Trends in ecology and evolution**, 20(7): 402-409.

Woodward, G. and Hildrew, A. G. (2002) The impact of a sit and wait predator: separating consumption and prey emigration. **Oikos**, 99: 409-418.

Yodzis, P. (1981) The stablility of real ecosystems. **Nature**, 289: 674-686.

4.7 Tables and figures

Table 4. 1 Grazer species density in experimental treatments. Size classes have mean grazer body length shell height +/- 1 SE in brackets).

Grazer	Size class (mean +/- 1 SE)	Density
Agapetus sp.	Small (1.17 +/- 0.34)	20
	Medium (2.14 +/- 0.46)	13
	Large (3.18 +/- 0.75)	9
Serratella ignita	Small (3.47 +/- 0.63)	4
	Medium (5.85 +/- 0.57)	3
	Large (7.76 +/- 0.54)	2
Radix balthica	Small (4.17 +/- 0.91)	3
	Medium (8.07 +/- 0.56)	2
	Large (10.82 +/- 1.08)	1

Table 4. 2 Algal size class categories based on algal cell lengths from a sub-sample of algae measured from experimental biofilms.

Algal size category	Size range (µm)
1	< 5
2	5 – 10
3	11 - 15
4	16 - 20
5	21 - 25
6	26 - 30
7	31 - 40
8	41 - 50
9	51 - 60
10	61 - 75
11	76 - 90
12	91 - 109
13	110 - 150
14	> 150

Table 4. 3 Results of nested ANOVA, testing differences between estimated per capita grazer interactions on algal AFDM and Chlorophyll *a*. A two factor design was used where body size was nested within grazer species.

Source of variation	**SS**	**df**	**MS**	**F - Ratio**	***P***
DI [AFDM]					
Species	0.083	2	0.042	64.996	< 0.001
Size(Species)	0.086	6	0.014	22.362	< 0.001
Error	0.022	35	0.001		
Total	0.247	44			
DI [Chlorophyll *a*]					
Species	0.161	2	0.081	46.080	< 0.001
Size(Species)	0.215	6	0.036	20.439	< 0.001
Error	0.061	35	0.002		
Total	0.494	44			

Table 4. 4 Results of principal components analysis: Eigenvalues for axes 1 to 4 and cumulative percentage variance of species data represented on each axis

Axes	**1**	**2**	**3**	**4**	**Total variance**
Eigenvalues	0.592	0.088	0.073	0.062	1
Cumulative percentage variance of species data	59.2	68	75.3	81.4	

Figure 4. 1Mean + / - 1SE grazer body lengths for each treatment. Different letters show significant differences between size classes within each grazer treatment (but not across grazer treatments).

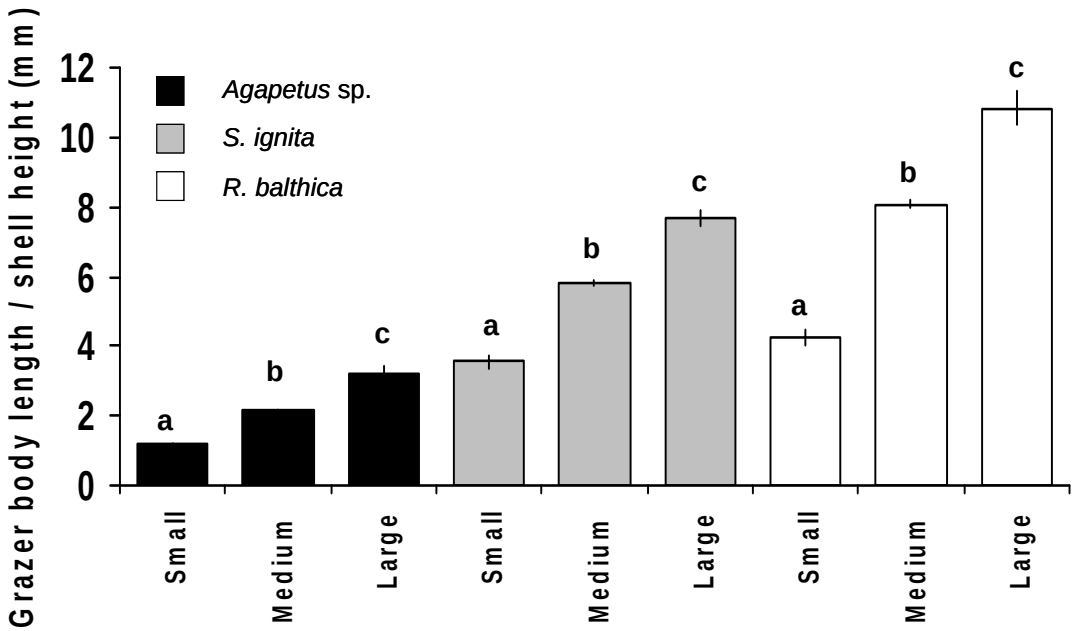


Figure 4. 2 Mean +/- 1 SE per capita interaction strength across grazers for a) AFDM and b) Chlorophyll *a*.. Means not significantly different have the same letter. Significant differences are represented by different letters.

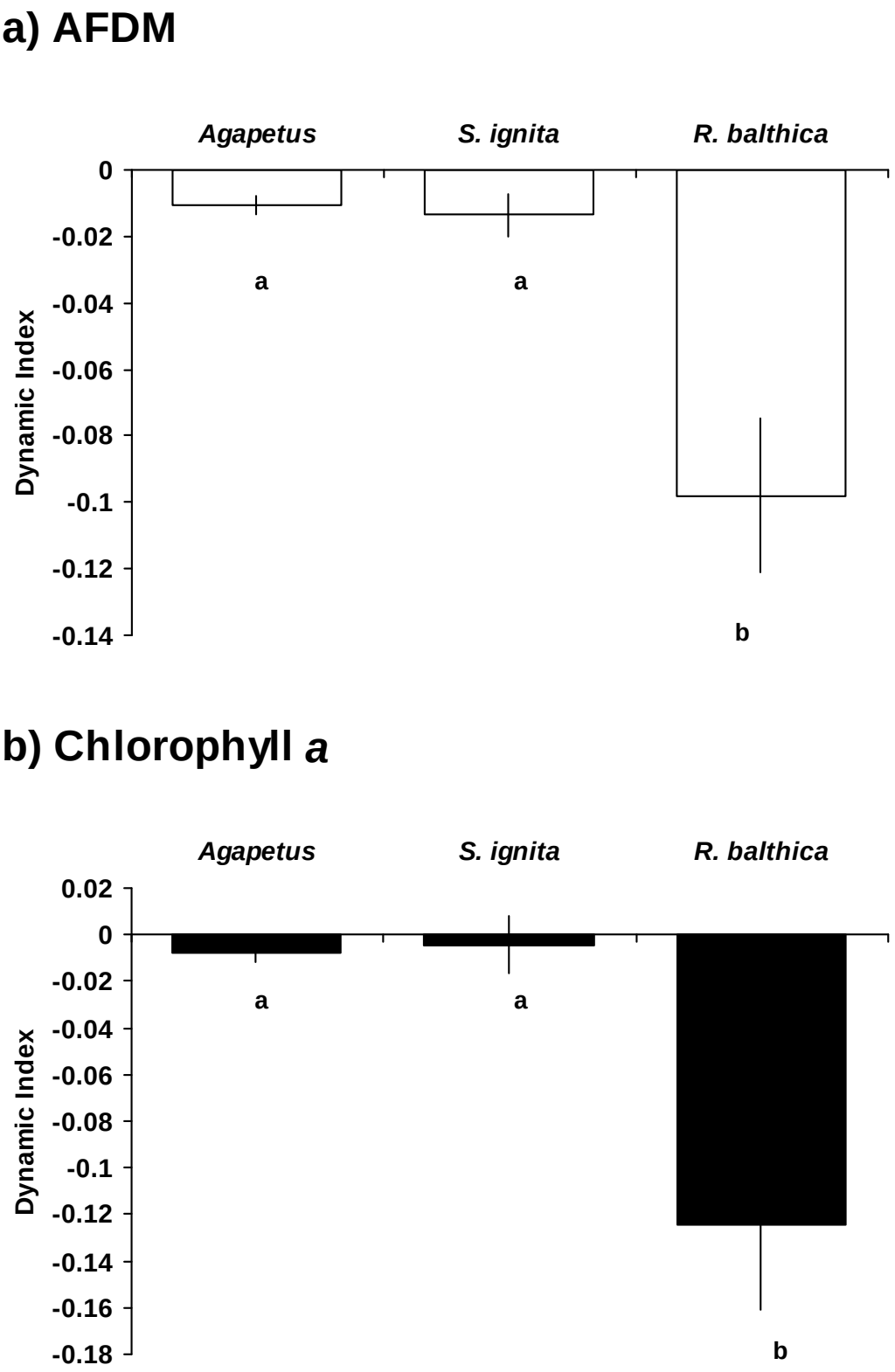
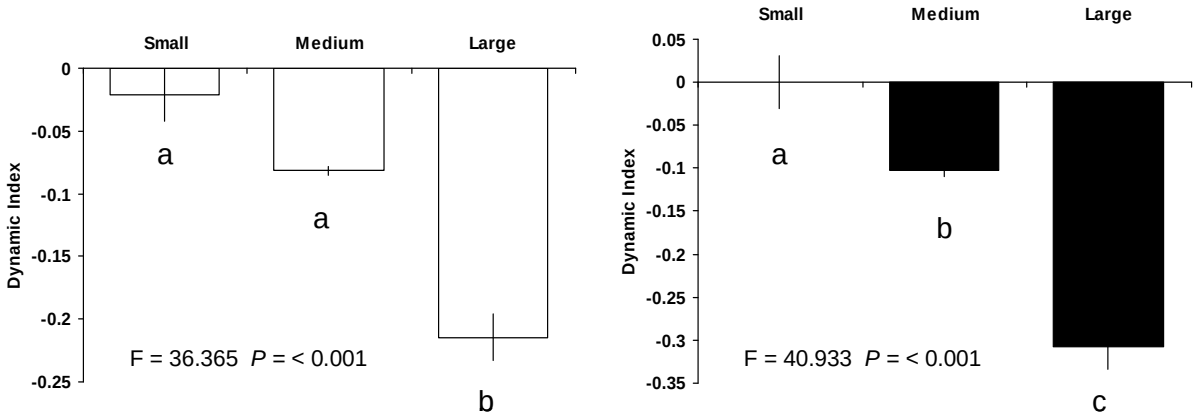
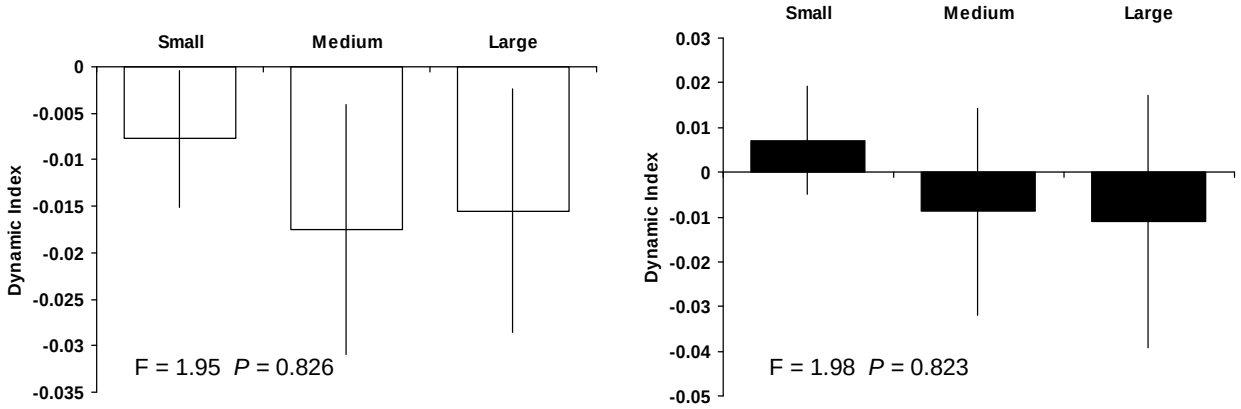


Figure 4. 3 Mean +/- 1 SE per capita interaction strengths (Dynamic Index) of a) *Radix balthica*, b) *Serratella ignita* and c) *Agapetus* sp. on biofilm AFDM (white bars) and Chlorophyll *a* (black bars) for small, medium and large body sizes. Significant differences found by Tukey's pairwise comparisons are indicated by different letters.

a) *Radix balthica*



b) *Serratella ignita*



c) *Agapetus* sp.

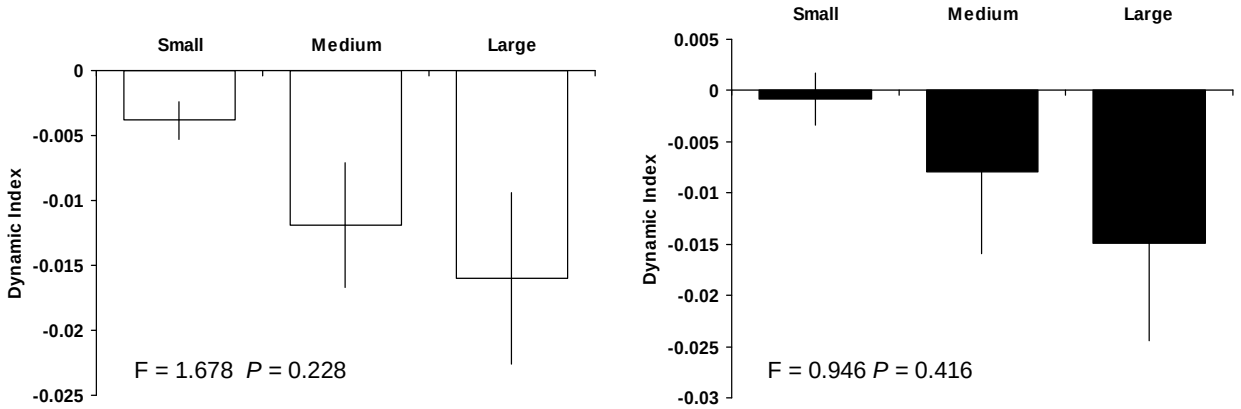
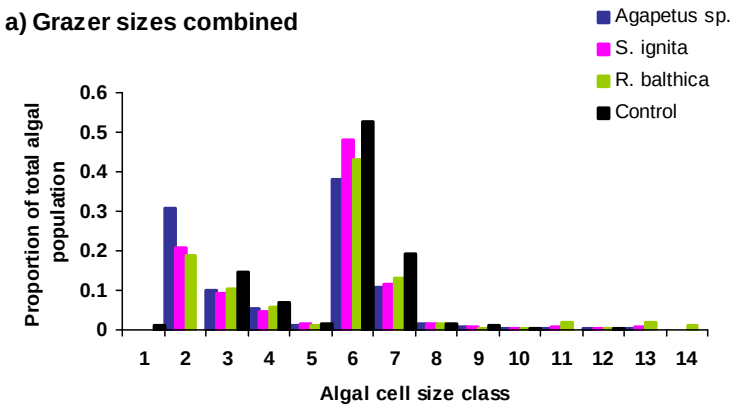
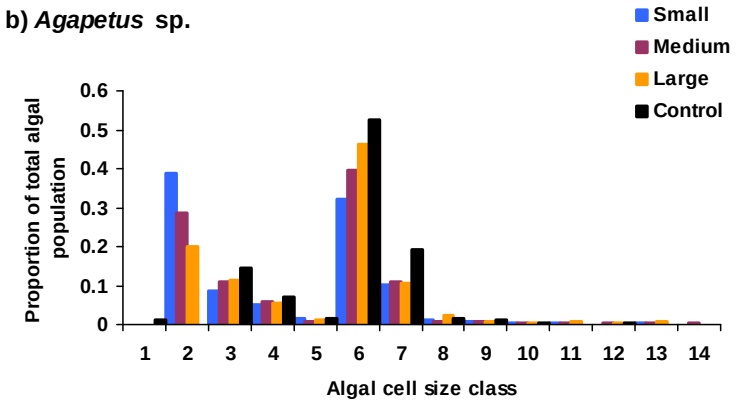
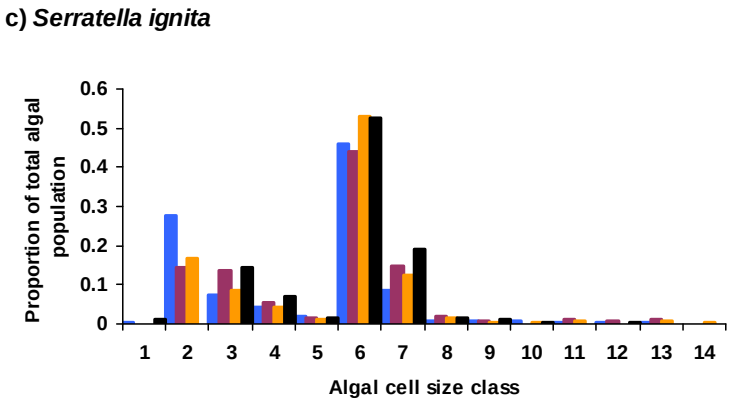


Figure 4. 4 Algal size distributions grazers grouped into a) small, medium and large, b) Agapetus sp., c) Serratella ignita and d) Radix balthica.







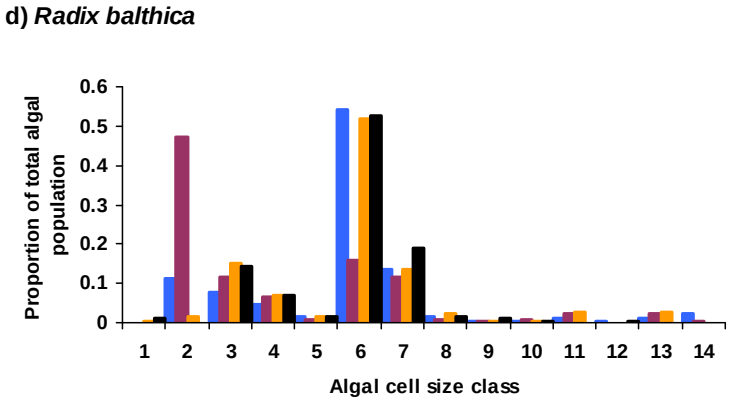


Figure 4. 5 Ordination plot of the first two axes of the PCA. Arrows represent size classes of algae.

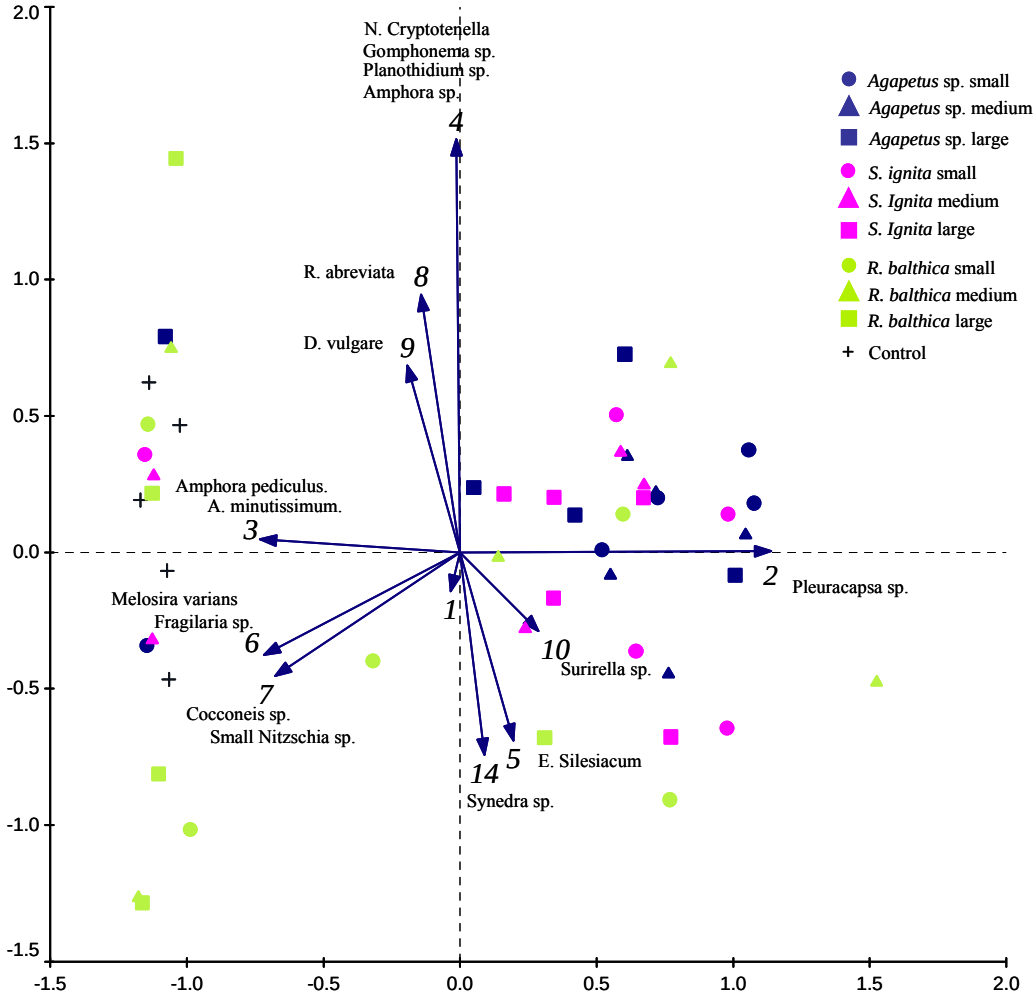
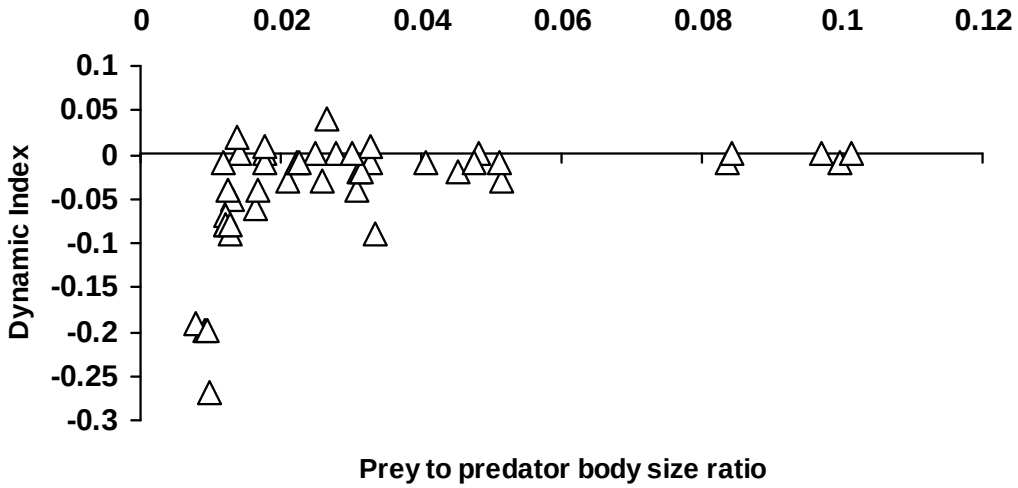
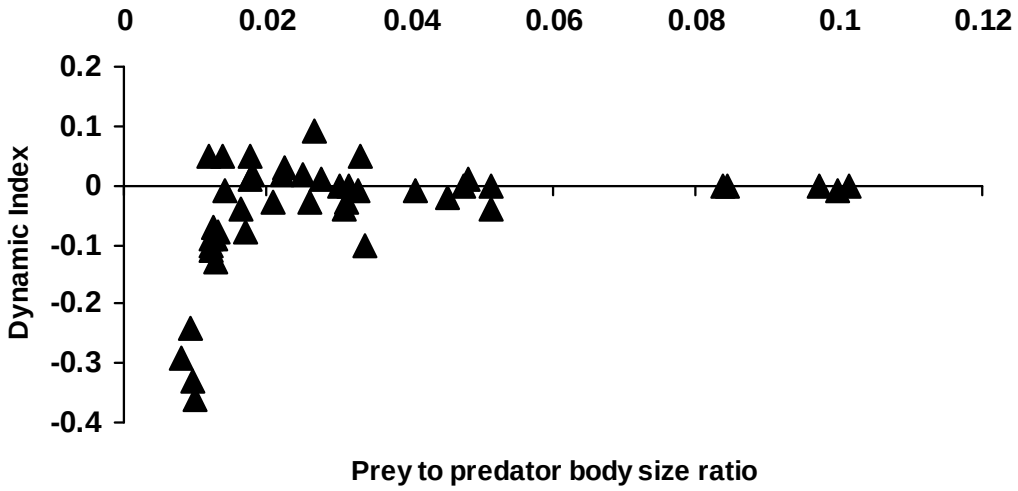


Figure 4. 6 Relationship between predator (Grazer) / prey (algae) body size ratios and Dynamic Index for a) AFDM and b) Chlorophyll *a*. Predator body size decreases as the ratio increases, i.e. grazers and their algal prey are move closer together in size.

a) AFDM



b) Chlorophyll *a*



CHAPTER 5 - INTERACTION STRENGTH AND COMPETITION AMONG THREE GASTROPOD MOLLUSCS

5.1 Summary

Using experimental mesocosms competitive interactions were investigated between three species of gastropod mollusc (*Radix balthica*, *Theodoxus fluviatilis* and *Bithynia tentaculata*) and a diverse algal biofilm. Intraspecific interactions of single species and interspecific interactions between paired species combinations and all three snails were tested. Results indicate that *R. balthica* was inhibited by the presence of competing snail species, particularly *T. fluviatilis*. No evidence of snail facilitation was found, although high levels of variability for the three snail treatment suggest complex interactions occurred, beyond the scope of this study.

5.2 Introduction

5.2.1 *Snails – important grazers?*

Grazers are important members of ecological communities. They assimilate the products of primary production, transferring resources to higher trophic levels, from all ecosystems including freshwater benthic communities (Allan, 1995). Their impact on lotic algal biofilms contributes to the level of energy transferred along the food chain and is therefore important in food web dynamics (Jordan and Lake, 1996; Keldsen, 1996; McNeely et al., 2007). Within this habitat grazing is employed by a diverse suite of organisms that originate from a wide range of taxonomic orders. Key grazers associated with the benthos include, in decreasing order according to their overall effect on biofilms, caddisfly larvae, gastropod molluscs and mayfly larvae; and the relative importance of snails in comparison to other grazers has been studied extensively (Feminella and Hawkins, 1995; Gresens, 1995; Alvarez and Peckarsky, 2005; Holomuzki and Biggs, 2006). However, there appear to be few studies examining the relative importance of different snails as grazers (Bronmark et al., 1991; Chase et al., 2001), making comparisons across species difficult. This is despite the fact that most snail species in the UK and Europe are either predominantly grazers or combine grazing with deposit feeding and to a lesser extent filter feeding (Moog, 1995, Tachet et al., 2000). Additionally, snail life cycles are not as discrete as those of insects, with multiple cohorts overlapping throughout the year (Macan, 1994), further increasing pressure on resources. Therefore, wherever snail species occur together in natural systems, competition between them for algal resources is expected to be considerable if resources are limiting (Begon et al., 1996). Consequently, exploring

snail species interactions, and particularly in Northern European areas such as the UK where they are abundant is paramount to understanding algal biofilm depression in natural systems where several snail species may co-exist.

5.2.2 *Snail competition for algal resources*

Variation in algal depression can be associated with differences in grazer mouthparts, feeding mode, or locomotion, such as crawling versus swimming. Such differences may be clearly apparent in many insect grazers but differences between snails are less distinct (Tachet et al., 2000). Consequently, niche overlap for resources between snail species may in fact be higher than for snails competing with grazers from other taxonomic groups (Brown, 1982). This could lead on to higher competitive interactions between species, especially if resources are limiting (Kohler, 1992; Tall et al., 2006) or spatial heterogeneity is low (Pacala and Roughgarden, 1982; Fletcher and Underwood, 1987; Robson and Barmuta, 1998). Resource competition is most likely to occur in less productive systems where primary production is limited (Abrams, 1988; McNeely et al., 2007). Under such extreme circumstances grazer populations are restricted by algal availability, i.e. there are clear bottom-up effects (Steinman, 1996; Feminella & Hawkins, 1995). In productive systems where primary production is the key source of organic carbon, snail species may experience a greater degree of interference competition because the densities of grazers are higher (e.g. Hart and Robinson, 1990; Munoz et al., 2000; Rosemond et al., 2000). This may be mediated by habitat space rather than resource density *per se*, i.e. grazers compete for access to resources (Wilson et al., 1999). This will include direct interactions where, for example one snail directly restricts the impact of another snail, or indirect

interactions where two individuals are linked indirectly through either of their impacts on a third animal or an algal type (see Liess and Hillebrand, 2004 for a review). Interference competition can either be among snails of the same species (intraspecific) or between species (interspecific), and is likely to be mediated by behaviour e.g. the feeding behaviour of one snail impairs the feeding behaviour of another (for further information see the review on behaviourally mediated interactions by Werner, 1992). For intraspecific interference there is likely to be some degree of temporal cohort separation, thus partitioning niche overlap into size classes (Werner and Gilliam, 1984), and individuals are also likely to disperse widely when eggs masses hatch (Bilton et al., 2001). Comparatively, interspecific interference competition will be prevalent whenever different snail species are in close proximity, and indeed interspecific competition has been found to frequently cause reduced performance among co-occurring grazing competitors (Kohler, 1992; Gresens, 1995; Lamberti et al., 1995; Alvarez and Peckarsky, 2005; Holomuzki and Biggs, 2006), particularly as densities increase (Steinman, 1991; Boaventura et al., 2003). Establishing snail interactions, whether inter- or intraspecific, is important in assessing impacts of snail communities on algal resources.

5.2.3 *Chalk stream snail communities*

Biofilms in chalk stream systems are highly diverse and usually dominated by calcareous crusts and diatomaceous filaments (Ledger et al., 2008 and chapter 3). Development of the biofilm is also unrestricted, occurring in shallow nutrient rich waters typical of lowland chalk rivers. Thus competition among snails is most likely to be characterised by interference competition in this habitat, because resources are

not limited by nutrient supply (see chapter 2). Previous work indicates that three snail species tested in isolation; *Radix balthica*, *Bithynia tentaculata* and *Theodoxus fluviatilis* depress algal biofilms differently depending on the dominant type of algae present (calcareous crusts or diatom filamentous mats, chapter 3). How effectively these snails would graze together has not been tested for a combined algal biofilm containing both crusts and mats. Compared to mats, crusts were found to be relatively unpalatable for each species; *T. fluviatilis* and *B. tentaculata* only exerted weak impacts on biomass and *R. balthica* failed to alter biomass (chapter 3). Furthermore, *R. balthica* was more successful at mat removal than the other snails. It is unclear whether combining snail species would induce a weaker (implying interference) impact on biofilms than expected if individuals behaved independently of one another (a simple additive effect).

5.2.4 *Research aims*

The objective of this experiment was:

1) To identify, using the dynamic index (see previous chapters), if snails separately and in combination exerted the same impacts on the AFDM, chlorophyll *a* and cell abundance of algal biofilms. In addition, the magnitude of the different measures of impacts (i.e. dynamic indices for AFDM, chlorophyll *a* and cell counts) was compared. The impact of treatments on the cell abundance of major algal groups was also compared. The relationship between per capita impact (AFDM and chlorophyll *a*) and the proportion of diatom and *G. incrustans* cells was examined.

2) To assess the evidence for inter- and intra specific competition between the snails (predominantly interference as resources were not limiting). It was expected that if no

competition occurred (or if inter- and intra specific competition had exactly the same magnitude), the impacts of the snails in single species treatments would be cumulative and thus would be equal to the impacts of the snails in combinations of species; i.e. no discernible evidence for competition occurred when:

DI (snail A) + DI (snail B) = DI (snails A and B together)

If interspecific competition occurred, and was stronger than intraspecific effects, the impacts on the biofilm in combined species treatments should be less than the sum of the impacts of single species treatments; i.e. interspecific competition occurred when:

DI (snail A) + DI (snail B) > DI (snails A and B together)

However, if intraspecific competition occurred, and was stronger than interspecific effects, or if some form of interspecific facilitation occurred, the sum of the impacts of single species treatments should be less than the impacts of combined treatments; i.e. intraspecific competition/interspecific facilitation occurred when:

DI (snail A) + DI (snail B) < DI (snails A and B together).

5.3 Methods

5.3.1 *Experimental design*

The impact of intra- and interspecific competition on algal resource depression among three snail species (*R. balthica*, *B. tentaculata* and *T. fluviatilis*) was investigated using an experimental mesocosm approach (see chapter 2 for a description of mesocosms) between June and September 2006 in the FBA fluvarium. A factorial analysis with seven levels was used: single species treatments (three levels); all possible paired combinations (three levels); and three snails together (one level). Each snail treatment and an ungrazed control were replicated five times, in five experimental blocks tethered to the north fluvarium channel and assigned to mesocosms within blocks at random.

Algal biofilms were allowed to colonise unglazed ceramic tiles (2.5cm x 2.5 cm) in bank side experimental channels over a three month period. During algal colonisation tiles were disturbed occasionally to encourage growth of both *G. incrustans* crusts and diatom assemblages.

Individual tiles were randomly distributed across experimental enclosures. The densities and shell sizes of snails used in treatments are given in table 5.1. At the end of the experimental period (70 hours) algae were removed and processed as described in chapter 2.

5.3.2 *Data analysis*

Per capita treatment impacts on algal biomass (AFDM and Chlorophyll *a*) and algal abundance (total cell counts) were calculated using the Dynamic Index (see previous chapters for a full description). Data tested for normality and homogeneity of variance (the Anderson-Darling and two-tailed Levene tests respectively, $\alpha = 0.05$) met the parametric assumptions of ANOVA. One-way ANOVA ($\alpha = 0.05$) was used to compare differences in per capita impact on biomass (AFDM and Chlorophyll *a*) and algal abundance (total cell counts) across the 7 snail treatments and control. Further one-way ANOVAs tested for differences among the three DI measures of per capita impacts on algal biomass and abundance within snail treatments. Per capita impacts on AFDM, Chlorophyll *a* and cell abundance were tested against the null hypothesis of no impact using t-tests with a null mean DI value of zero (2 tailed $\alpha = 0.05$). Mean abundances of major algal groups and common diatom groups were examined across snail treatments using one-way ANOVAs. Differences between treatment means were examined using Tukey's pairwise comparisons ($\alpha = 0.05$). The relationship between proportions of dominant algal taxa (*G. incrustans* and total diatoms) and per capita interaction strength on algal biomass (DI AFDM and Chlorophyll *a*) across the whole data set was tested using Pearson's correlation ($\alpha = 0.05$). Per capita impacts (dynamic index) on *G. incrustans* and total diatom abundance across treatments were compared across treatments using one-way ANOVAs ($\alpha = 0.05$).

To estimate competition, the expected impact of a snail combination under the assumption of simple additivity (i.e. no facilitation or interference) was calculated. This was achieved by summing the impacts of snails in single treatments for each block, thus yielding five replicate expected values for each measure of impact

(AFDM, chlorophyll *a* and cell abundance). This was calculated for the three two-snail combination treatments and the one three-snail combination treatment. Expected values were then compared to the actual observed values for each combination treatment using paired t-tests ($\alpha = 0.05$, pairing within blocks).

5.4 Results

5.4.1 *Treatment effects on Algal biomass (AFDM & Chlorophyll a) and cell counts*

On the whole, the seven experimental treatments depressed the algal biofilm, giving negative Dynamic Index values (figure 5.1), and particularly in treatments containing *R. balthica*. However, across the snail treatments, there were no significant differences in any of the three per capita interaction strength estimates of biofilm depression (table 5.2). Despite this, non-statistically significant patterns were evident in the data (figure 5.1), where; 1) combined treatments containing *R. balthica* appeared to depress algal biomass more, i.e. larger negative dynamic index values for AFDM and Chlorophyll *a*, 2) *R. balthica* had a clear negative impact on total cell counts, *T. fluviatilis* exerted a slight negative impact, and all other treatments showed little or no effect and 3) variability within treatments was high but was greatest in the three species combined treatment (figure 5.1).

5.4.2 *AFDM, Chlorophyll a and algal cell counts: differences within a treatment*

Effects of snails on biofilm biomass and total cell abundance, expressed as dynamic indices, did not differ significantly within treatments (table 5.3). Snails were statistically impacting biomass and abundance equally. However, visible patterns in the dynamic index within treatments indicated snails were probably altering algal biomass differently to abundance (figure 5.1). Effects varied depending on snail treatment and the type of biofilm constituent depressed. Single snails reduced algal biomass (AFDM and Chlorophyll *a*) and cell abundance similarly, e.g. strong

depression of all three algal parameters for *R. balthica*; weak depression for *T. fluviatilis* and no effect for *B. tentaculata*. Combined treatments reduced biomass with relatively little change in algal abundance (figure 5.1).

5.4.3 *Magnitude of interaction: impacts significantly different from zero*

The t-tests detected relatively few interactions where the dynamic index was significantly different from zero which, depended on the snail species and the particular response variable examined. Chlorophyll *a* was significantly depressed (negative DI) in three treatments (*R. balthica*; *R. balthica* / *B. tentaculata*; and *R. balthica* / *T. fluviatilis*). AFDM was also strongly depressed (negative DI) in *R. balthica* and *R. balthica* / *B. tentaculata*. Cell counts were strongly affected by *R. balthica* only (table 5.4). Biofilm depression was driven by *R. balthica* alone and pairwise combinations containing *R. balthica*. Effects appeared to decline from *R. balthica* on its own to *R. balthica* / *B. tentaculata* and *R. balthica* / *T. fluviatilis* (figure 5.1.).

5.4.4. *Snail impacts on the composition of algal communities*

Snail grazing weakly affected *G. incrustans* abundance (figure 5.2.a.). A difference between treatment means was detected by a one-way ANOVA ($F = 2.378$, $P = 0.045$). However, Tukey's pairwise comparisons failed to detect differences between means at this level of significance. In this case snail treatment means could not be interpreted without some degree of caution and the null hypothesis; that there was no significant difference in *G. incrustans* abundance across treatments, was accepted. Despite a lack

of power to detect differences in the data, weak patterns were evident for *R. balthica* and *T. fluviatilis* single snail treatments, which appeared to depress *G. incrustans* abundance relative to controls. No significant difference in total diatom cell numbers occurred across treatments (F = 1.543, *P* = 0.189). Nevertheless, a non-significant pattern was observed for *R. balthica*, which depressed diatom cells considerably (figure 5.2.b). Treatment effects on the less abundant blue / green alga *Pleurocapsa* spp. were clear (F = 3.278, *P* = 0.01). Cell abundance increased for all combined snail treatments, except in the *B. tentaculata* / *T. fluviatilis* combined treatment where abundances remained low. Furthermore, low numbers of *Pleurocapsa* spp. occurred in ungrazed control and single snail treatments (figure 5.2.c)

5.4.5. *Snail impacts on the composition of Diatom species*

Only a single diatom group varied significantly across snail treatments; small *Navicula* spp. / *Achnanthes* spp (F = 4.608, *P* = 0.001). Tukey's pairwise comparisons showed that *R. balthica* and *T. fluviatilis* single treatments reduced these small adnate diatoms more than *R. balthica* / *B. tentaculata* and three snails combined. Nevertheless, no treatment differed significantly from controls in the abundance of this diatom group (figure 5.3). For all other diatom groups, snail treatments did not induce statistically different impacts from each other or controls. However, patterns in diatom depression were evident. Snail treatments regularly induced reductions in abundance compared to controls. Particularly in the adnate understory species (e.g. *A. minutissimum*, *Amphora* spp. and to a lesser extent *Cocconeis* spp.) and the motile species attached to them (e.g. *Navicula* spp. and *Nitzschia* spp.). In addition, high variance within diatom groups was evident, from the

size of the standard error bars, particularly for the overstory chain-forming diatoms, such as, *Melosira varians* and to a lesser extent *Fragilaria* spp. (figure 5.3).

5.4.6. *Per capita interaction strength (DI) and the proportion of G. incrustans versus diatom cells*

There was no correlation between proportional abundance of *G. incrustans* and per capita impacts on algal biofilm (AFDM and Chlorophyll *a*) (table 5.5). Depression of AFDM and Chlorophyll *a* both remained the same as the proportion of *G. incrustans* cells increased (figure 5.4.a). The opposite was observed for the proportional abundance of total diatom cells, which correlated well with per capita impacts on AFDM and Chlorophyll *a* (table 5.5). As the proportion of diatom cells increased the dynamic index increased, from negative values towards zero, i.e. grazer impact decreased (figure 5.4.b).

5.4.7 *Per capita impacts on G. incrustans and total diatom cells*

Per capita interactions on *G. incrustans* and total diatoms abundance (dynamic index using cell counts) were relatively weak (maximum values of -0.0015 and -0.0025 respectively). However the dynamic index did not differ between treatments for either *G. incrustans* or total diatoms cells. Nonetheless, treatment impacts on diatoms were consistently negative while *G. incrustans* incurred a mixture of positive and negative impacts (figure 5.5). Combined grazer treatments (except *R. balthica* / *T. fluviatilis*) were particularly poor at depressing *G. incrustans*. *R. balthica* and *T. fluviatilis* were

better at depressing *G. incrustans*, but their combined impact was weaker than independent additive impacts.

5.4.8 *Comparison of expected and observed impacts in combination treatments*

The observed values for per capita impacts on the biofilms in snail combination treatments did not differ significantly from expected values (table 5.6). However there was a clear trend for lower per capita impacts in the observed values than in the expected values (figure 5.6). This effect was marked for cell abundance impacts in all snail combinations. A similar pattern, although weaker, was observed for AFDM and chlorophyll *a*, and in particular for the *B. tentaculata*/*T. fluviatilis* combination. This obvious discrepancy supports the assumption that interference occurred when snails were combined in treatments.

5.5 Discussion

5.5.1 *Snail impacts on biofilm biomass and assemblage parameters*

Treatment impacts were subtle, with similar impacts on algal biofilms across snail treatments. Snails are important grazers that reduce biofilm biomass and change algal composition and assemblage structure (Lamberti et al., 1989; Underwood et al., 1992; Feminella and Hawkins, 1995; Steinman, 1996; Wilson et al., 1999; Chase et al., 2001). They are often compared to other orders of grazing invertebrates particularly caddisfly and mayfly larvae, however these groups often exert stronger and weaker impacts, respectively, compared to snails (Chapter 2; Chapter 3; Lamberti et al., 1995; Holomuzki and Biggs, 2006). Grazer characteristics such as mouthpart morphology, feeding mode and mobility contribute to the difference in grazer effect magnitude (Alvarez and Peckarsky, 2005). Consequently, the probability of detecting interspecific differences is higher when comparing species across higher taxonomic groups (Hill and Knight, 1988; Feminella et al., 1989; DeNicola et al., 1990; McNeely et al., 2007). Moreover, studies comparing differences between species within the same taxonomic group are rare in the literature (Barnese et al., 1990; Wellnitz and Ward, 1998). Of those available, most focus exclusively on caddisfly and mayfly species, with few addressing multiple snail effects (Lamberti and Resh, 1983; Underwood, 1992; Chase et al., 2001). In these studies snail impacts are typically described in terms of changes in algal assemblage structure and relative population abundance rather than biomass reduction (Jordan and Lake, 1996).

The lack of power to detect significant grazer impacts is possibly an artefact of similarities in snail feeding mode and morphology (Brown, 1982). Resource partitioning coupled with discrete niche space all contribute to co-existence and reduced competition among taxonomically similar species (Wilson et al., 1999). Consequently, although patterns were detected in this experiment the variability within the data was not easily attributable to the particular treatments analysed, in part due to similarities in snail grazing habits (Barnese et al., 1990). Intricate grazer effects at the algal taxon level (high variability) prevent firm conclusions from being drawn, with the exception that across treatments snails were reducing some algal taxa but not others. This was predominantly illustrated by *R. balthica* impacting total diatoms and *G. incrustans*.

Comparisons of diatom abundance also produced an unclear picture across treatments. Considerable variation in the depression of *M. varians* is probably a consequence of the chain-forming morphology of this species. *Melosira varians* forms long filaments that contribute to a complex biofilm physiognomy (Krammer and Lange-Bertalot, 1991; Kelly, 2000). These chains of algae reach a size and location within the biofilm which protects them from some grazers but not others (Munoz et al., 2000), such as R. *balthica* which negatively affected cell numbers. This may have been driven by relative body size, consistent with the impacts of large *R. balthica* observed in chapter 4. Low-level adnate cells, such as *A. minutissimum*, *Amphora* spp. and *Cocconeis* spp. are depressed consistently by all treatments as they are easily cropped by the snail's radula as it moves across the substrate. Evidence suggests that these smaller adnate and prostrate forms are susceptible to snail grazing (Underwood, 1992; Jordan and Lake, 1996; Holomuzki and Biggs, 2006).

5.5.2 *Competition – facilitation, inhibition or intraspecific?*

Single snail species impacts were modified upon the addition of another species. The species in combinations generally exerted weaker effects than the additive effects of the constituent species in isolation. Consequently, this pattern suggests some degree of interspecific competition was occurring in the combined treatments. Species experiencing niche overlap ultimately compete either through consumption (resource competition e.g. Kohler, 1992) or by interference (e.g. Gresens, 1995). It is unlikely that resources were limiting in this experiment, as no treatment significantly reduced algal cell abundance from controls. Consequently, it is more likely competition between snails was mediated by interference (Brown, 1982). This could have been direct, for example the highly mobile *R. balthica* disturbing the more sedentary *B. tentaculata* and *T. fluviatilis*. Alternatively, indirect competition effects through the impact of one snail on an intermediate species may have influenced feeding behaviour, for example in the three snail treatment. However indirect effects in grazers are often mediated by a shared algal prey, not a trophic rival (Feminella and Hawkins, 1995; Begon et al., 1996; Wilson et al., 1999), therefore the intermediate species was most probably an algal species. Thus, in combined species treatments where a distinct pattern of weaker effects was observed, direct snail interference is most likely to have caused the reduced effect on biofilms. Evidence of an indirect interference via an algal intermediate was not clearly discernable from this study, i.e. no clear patterns in algal abundance were detected and therefore could not be linked to snail performance.

It is possible that a combination of direct and indirect interactions were occurring. Direct interference between snails is likely to induce a behavioural response in one or

all species (Bronmark et al., 1992; Brown et al., 1994; Singer and Bernays, 2003). *R. balthica* had the greatest impact as a single species but was less successful combined with a second and third species. However, in the presence of two heterospecifics *R. balthica* appears to be released, reducing biofilm biomass similarly to single *R. balthica*, and perhaps this was due to interference between the two other snails. However, variation within the three species treatment was high, possibly due to a complex mixture of direct and indirect interference between the three snail species.

One algal species (*Pleurocapsa* spp.) appeared to benefit from combined snail species treatments but not single species treatments. In isolation from other species, snails are free to consume the biofilm as they encounter it, i.e. they show little selectivity and consume all algae proportionally (Lamberti et al., 1989; Liess and Kahlert, 2007) In the presence of competitors selection of resources could lead to an increase in abundance of non-selected items e.g. less nutritious blue / green *Pleurocapsa* spp. (Brown et al., 1997). The increasing abundance of *Pleurocapsa* spp. suggests competitive interactions between snails promote the growth of this alga.

5.5.3 *Gongrosira incrustans or diatoms as drivers of grazer impact*

Although *Gongrosira incrustans* was abundant in the biofilm, its proportional abundance had little influence on per capita impacts on algal biomass (DI AFDM and Chlorophyll *a*). As *G. incrustans* grows it forms a tough calcareous matrix which is less easily grazed from the substrate (Chapter 4; Munoz et al., 2000; Elser et al., 2005). The removal of this alga from single snail treatments (specifically *R. balthica* and *T. fluviatilis*) but not combined treatments is further evidence of snail selectivity in the presence of competing grazers. When isolated from competitors, *R. balthica*

and *T. fluviatilis* consume the biofilm indiscriminately decreasing tough *G. incrustans* cells along with diatoms and other algae. But, in the presence of other snails depression of this less easily grazed species is restricted in favour of more palatable resources, namely diatom species (Brown et al., 1997). The relationship between the proportion of diatom cells and per capita impacts illustrates the important role diatoms play in the interaction between snail grazers and the biofilm (Bergey, 1995; Jordan and Lake, 2006; Tall et al., 2006).

5.5.4 *Experimental improvements*

This experiment was aimed at detecting interspecific competition between grazing snail species. The main factor tested was comprised of a series of levels that represented different scenarios of competition. This design did not isolate the variation in the data sufficiently to draw solid conclusions about the effects of intra- and interspecific competition between grazing snails and algal biofilms. Indisputable design faults were apparent once the experiment had been conducted. Firstly, the magnitude of intraspecific competition was not determined in the single snail treatments. Consequently, as snail densities for each combined treatment were not held constant with the single treatment, the effects of density on intraspecific competition were unknown. A series of experiments measuring intraspecific competition over a range of snail densities, for each species, would enable the experimenter to assign density mediated intraspecific competition parameters to the expected versus observed model. Secondly, the tri species treatment failed to incorporate intraspecific competition within the model (only 1 individual of each species was utilised). Again, testing a range of densities would make the model more

robust to changes in intraspecific competition. Therefore a series of factorally designed experiments with more than one factor, e.g. one experiment investigating single snail species and grazer density, another investigating pairwise combinations and densities etc, may prove to be a better design for studying these types of interaction.

The algal biofilms used in this experiment were manipulated to create a single algal resource with two distinct algal patch types; a lower *Gongrosira incrustans* dominated crust with an overstory of thick diatoms dominated by *Melosira varians* filaments. Combining patches into a single resource in this way perhaps constrained specific snail species access to particular algal components, and hence increase the variability within the data set. A clear solution could be to retain separation of the patch types and assess effects on the patches independently.

To simulate a wider spatial context, experimental work could be extended to in-stream exclusions/inclusions at the reach scale; indeed Taylor et al (2002) compared reach scale manipulations to mesocosm manipulations and found interaction strength measures to be three times greater at the reach scale. Further work could also study the influence of algal patchiness, as this is an important determinant of grazer impacts at small spatial scales (Kawata et al., 2001).

5.6 References

Abrams, P. A. (1988) Resource Productivity Consumer Species-Diversity - Simple-Models Of Competition In Spatially Heterogeneous Environments. **Ecology**, 69(5): 1418-1433.

Allan, J. D. (1995) **Stream ecology: Structure and function of running waters.** London: Chapman and Hall.

Alvarez, M. and Peckarsky, B. L. (2005) How do grazers affect periphyton heterogeneity in streams? **Oecologia**, 142(4): 576-587.

Barnese, L. E., Lowe, R. L. and Hunter, R. D. (1990) Comparative grazing efficiency of pulmonate and prosobranch snails. **Journal of the North American Benthological Society**, 9(1): 35-44.

Begon, M., Harper, J. L. and Townsend, C.R. (1996) **Ecology: individuals, populations and communities**. 3rd edition. London, Wiley Blackwell. 1068 pp.

Bergey, E. A. (1995) Local effects of a sedentary grazer on stream algae. **Freshwater Biology**, 33(3): 401-409.

Bilton, D. T., Freeland, J. R. and Okamura, B. (2001) Dispersal in freshwater invertebrates. **Annual Review Of Ecology And Systematics**, 32: 159-181.

Boaventura, D., Cancela De Fonseca, L. and Hawkins, S. J. (2003) Size matters: competition within populations of the limpet *Patella depressa*. **Journal of Animal Ecology**, 72: 435-446.

Bronmark, C., Klosiewski, S. P. and Stein, R. A. (1992) Indirect effects of predation in a freshwater benthic food chain. **Ecology**, 73(5): 1662-1674.

Bronmark, C., Rundle, S. D. and Erlandsson, A. (1991) Interactions Between Fresh-Water Snails And Tadpoles - Competition And Facilitation. **Oecologia**, 87(1): 8-18.

Brown, K. M. (1982) Resource Overlap And Competition In Pond Snails - An Experimental-Analysis. **Ecology**, 63(2): 412-422.

Brown, K. M., Carman, K. R. and Inchausty, V. (1994) Density-dependent influences on feeding metabolism in a freshwater snail. **Oecologia**, 99: 158-165.

Brown, M. R., Jeffrey, S. W., Volkman, J. K., et al. (1997) Nutritional properties of microalgae for mariculture. **Aquaculture**, 151: 315-331.

Chase, J. M., Wilson, W. G. and Richards, S. A. (2001) Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. **Ecology Letters**, 4: 304-312.

DeNicola, D. M., McIntire, C. D., Lamberti, G. A., et al. (1990) Temporal patterns of grazer-periphyton interactions in laboratory streams. **Freshwater Biology**, 23: 475-489.

Elser, J. J., Schampel, J. H., Kyle, M., et al. (2005) Response of grazing snails to phosphorus enrichment of modern stromatolitic microbial communities. **Freshwater Biology**, 50: 1826-1835.

Feminella, J. W. and Hawkins, C. P. (1995) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. **Journal of the North American Benthological Society**, 14(4): 465-509.

Feminella, J. W., Power, M. E. and Resh, V. H. (1989) Periphyton responses to grazing and riparian canopy in three Northern California coastal streams. **Freshwater Biology**, 22: 445-457.

Fletcher, W. J. and Underwood, A. J. (1987) Interspecific Competition Among Subtidal Limpets - Effect Of Substratum Heterogeneity. **Ecology**, 68(2): 387-400.

Gresens, S. E. (1995) Grazer Diversity, Competition And The Response Of The Periphyton Community. **Oikos**, 73(3): 336-346.

Hart, D. D. and Robinson, C. T. (1990) Resource Limitation In A Stream Community - Phosphorus Enrichment Effects On Periphyton And Grazers. **Ecology**, 71(4): 1494-1502.

Hill, W. R. and Knight, A. W. (1988) Concurrent Grazing Effects of 2 Stream Insects on Periphyton. **Limnology and Oceanography**, 33(1): 15-26.

Holomuzki, J. R. and Biggs, B. J. F. (2006) Food limitation affects algivory and grazer performance for New Zealand stream macroinvertebrates. **Hydrobiologia**, 561: 83-94.

Jordan, J. and Lake, P. S. (1996) Grazer-epilithon interactions in an Australian upland stream. **Marine And Freshwater Research**, 47(6): 831-835.

Kawata, M., Hayashi, M. and Hara, T. (2001) Interactions between neighboring algae and snail grazing in structuring microdistribution patterns of periphyton. **Oikos**, 92: 404-416.

Keldsen, K. (1996) Regulation of algal biomass in a small lowland stream: Field experiments on the role of invertebrate grazing, phosphorus and irradiance. **Freshwater Biology**, 36(3): 535-546.

Kelly, M. (2000) Identification of common benthic diatoms in rivers. **Field studies**, 9: 583-700.

Kohler, S. L. (1992) Competition and the structure of a benthic stream community. **Ecological Monographs**, 62(2): 165-188.

Krammer, K. and Lange-Bertalot, H. (1991) **Susswasserflora von mitteleuropa, Bd 2/1 - 2/5: Bacillariophyceae.** Gustav Fischer Verlang.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., et al. (1995) Influence Of Grazer Type And Abundance On Plant-Herbivore Interactions In Streams. **Hydrobiologia**, 306(3): 179-188.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., et al. (1989) Productive Capacity of Periphyton as a Determinant of Plant Herbivore Interactions in Streams. **Ecology**, 70(6): 1840-1856.

Lamberti, G. A. and Resh, V. H. (1983) Stream Periphyton and Insect Herbivores: an Experimental Study of Grazing by a Caddisfly Population. **Ecology**, 64(5): 1124-1135.

Ledger, M. E., Harris, R. M. L., Armitage, P. D., et al. (2008) Disturbance, patchiness and coexistence in freshwater algal communities. **Oecologia**, 155: 609-819.

Liess, A. and Hillebrand, H. (2004) Invited review: Direct and indirect effects in herbivore periphyton interactions. **Archiv Fur Hydrobiologie**, 159(4): 433-453.

Liess, A. and Kahlert, M. (2007) Gastropod grazers and nutrients, but not light, interact in determining periphytic algal diversity. **Oecologia**, 152(1): 101-111.

Macan, T. T. (1994) **A key to the British fresh- and brackish-water gastropods with notes on their ecology. Scientific publication No. 13.** Ambleside: Freshwater Biological Association.

McNeely, C., Finlay, J. C. and Power, M. E. (2007) Grazer traits, competition, and carbon sources to a headwater-stream food web. **Ecology**, 88(2): 391-401.

Moog, O. (ed.) (1995). **Fauna Aquatica Austriaca**. Vienna: Wasser Wirtschafts Kataster, Bundesministerium fur land- Forstwirtschaft.

Munoz, I., Real, M., Guasch, H., et al. (2000) Resource limitation by freshwater snail (Stagnicola vulnerata) grazing pressure: an experimental study. **Archiv Fur Hydrobiologie**, 148(4): 517-532.

Pacala, S. W. and Roughgarden, J. (1982) Spatial Heterogeneity And Interspecific Competition. **Theoretical Population Biology**, 21(1): 92-113.

Robson, B. J. and Barmuta, L. A. (1998) The effect of two scales of habitat architecture on benthic grazing in a river. **Freshwater Biology**, 39(2): 207-220.

Rosemond, A. D., Mulholland, P. J. and Brawley, S. H. (2000) Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients, and

herbivores. **Canadian Journal Of Fisheries And Aquatic Sciences**, 57(1): 66-75.

Singer, M. S. and Bernays, E. A. (2003) Understanding omnivory needs a behavioural perspective. **Ecology**, 84(10): 2532-2537.

Steinman, A. D. (1991) Effects of herbivore size and hunger level on periphyton communities. **Journal of phycology**, 27: 54-59.

Steinman, A. D. and Lamberti, G. A. (1996) "Biomass and pigments of benthic algae". In Hauer, F. R. and Lamberti, G. A. (ed.) **Methods in stream ecology.** London, Academic press. pp. 295-313.

Tachet, H., Bournaud, M., Richoux, P., et al. (2000) **Invertébrés d'eau douce: systématique, biologie, écologie. Formated for STAR partners by: Usseglio-Polatera P. (2003/04).** Paris: CNRS Editions.

Tall, L., Cattaneo, A., Cloutier, L., et al. (2006) Resource partitioning in a grazer guild feeding on a multilayer diatom mat. **Journal Of The North American Benthological Society**, 25(4): 800-810.

Taylor, B. W., McIntosh, A. R. and Peckarsky, B. L. (2002) Reach-scale manipulations show invertebrate grazers depress algal resources in streams. **Limnology and Oceanography**, 47(3): 893-899.

Underwood, G. J. C., Thomas, J. D. and Baker, J. H. (1992) An experimental investigation of interactions in snail-macrophyte-epiphyte systems. **Oecologia**, 91: 587-595.

Wellnitz, T. A. and Ward, J. V. (1998) Does light intensity modify the effect mayfly grazers have on periphyton? **Freshwater Biology**, 39: 135-149.

Werner, E. E. (1992) Individual Behavior And Higher-Order Species Interactions. **American Naturalist**, 140: S5-S32.

Werner, E. E. and Gilliam, J. F. (1984) The Ontogenetic Niche And Species Interactions In Size Structured Populations. **Annual Review Of Ecology And Systematics**, 15: 393-425.

Wilson, W. G., Osenberg, C. W., Schmitt, R. J., et al. (1999) Complementary foraging behaviors allow coexistence of two consumers. **Ecology**, 80(7): 2358-2372.

5.7 Tables and figures

Table 5. 1 Snail densities and shell height range (mm) for each treatment. (except *T. fluviatilis*, where shell width was used).

Treatment	Snail species	Density	Shell height/width mm
R	*R. balthica*	4	5.5 - 8.7
B	*B. tentaculata*	4	5.5 - 7.5
T	*T. fluviatilis*	4	4.3 - 5.9
R / B	*R. balthica*	2	5.4 - 8.0
	B. tentaculata	2	6.1 - 7.4
R / T	*R. balthica*	2	5.7 - 7.4
	T. fluviatilis	2	4.1 - 5.1
B / T	*B. tentaculata*	2	6.1 - 7.5
	T. fluviatilis	2	4.2 - 5.2
R / B / T	*R. balthica*	1	7.5 - 8.2
	B. tentaculata	1	8.1 - 8.9
	T. fluviatilis	1	4.5 - 5.2

Table 5. 2 Results of one-way ANOVA's comparing the three dynamic indices across snail grazer treatments.

Source of variation	**SS**	**df**	**MS**	**F**	***P***
DI [AFDM]					
Snail treatment	0.007	6	0.001	1.077	0.400
Error	0.029	28	0.001		
Total	0.060	35			
DI [Chlorophyll *a*]					
Snail treatment	0.009	6	0.002	1.280	0.298
Error	0.035	28	0.001		
Total	0.069	35			
DI [Total algal cell counts]					
Snail treatment	0.006	6	0.001	1.806	0.134
Error	0.015	28	0.001		
Total	0.167	35			

Table 5. 3 Results of ANOVA comparing per capita snail impacts on different estimate of algal biomass.

R = *Radix balthica*; **B** = *Bithynia tentaculata*; **T** = *Theodoxus fluviatilis*; **R/B** = *R. balthica* and *B. tentaculata*; **R/T =** *R. balthica* and *T. fluviatilis*; **B/T** = *B. tentaculata* and *T. fluviatilis*; and **R/B/T** = all three snail species. (DI Response = DI AFDM, Chlorophyll *a* and cell counts)

Source of variation	**SS**	**df**	**MS**	**F**	***P***
R					
DI Response	0.000	2	0.000	0.209	0.814
Error	0.008	12	0.001		
Total	0.036	15			
B					
DI Response	0.000	2	0.000	0.119	0.889
Error	0.006	12	0.000		
Total	0.006	15			
T					
DI Response	0.000	2	0.000	0.206	0.816
Error	0.011	12	0.001		
Total	0.016	15			
R / B					
DI Response	0.002	2	0.001	2.210	0.152
Error	0.006	12	0.001		
Total	0.016	15			
R / T					
DI Response	0.001	2	0.000	1.014	0.392
Error	0.006	12	0.000		
Total	0.015	15			
B / T					
DI Response	0.001	2	0.000	0.428	0.662
Error	0.010	12	0.001		
Total	0.013	15			
R / B / T					
DI Response	0.006	2	0.003	1.074	0.372
Error	0.036	12	0.003		
Total	0.057	15			

Table 5. 4 t-test results for AFDM, Chlorophyll *a* and total cell counts across snail treatments.

R = *Radix balthica*; **B** = *Bithynia tentaculata*; **T** = *Theodoxus fluviatilis*; **R/B** = *R. balthica* and *B. tentaculata*; **R/T =** *R. balthica* and *T. fluviatilis*; **B/T** = *B. tentaculata* and *T. fluviatilis*; and **R/B/T** = all three snail species. Significant treatments are in bold.

	AFDM Test Value = 0					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% confidence interval of the difference**	
					Lower	**Upper**
R	-3.166	4	**0.034**	-0.040	-0.075	-0.005
B	-0.589	4	0.588	-0.005	-0.029	0.019
T	-1.097	4	0.334	-0.012	-0.041	0.018
R / B	-2.793	4	**0.049**	-0.031	-0.062	0.000
R / T	-2.274	4	0.085	-0.029	-0.064	0.006
B / T	-1.608	4	0.183	-0.018	-0.049	0.013
R / B / T	-1.746	4	0.156	-0.047	-0.121	0.027

	Chlorophyll *a* Test Value = 0					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% confidence interval of the difference**	
					Lower	**Upper**
R	-6.290	4	**0.003**	-0.049	-0.071	-0.027
B	0.162	4	0.879	0.001	-0.023	0.026
T	-1.043	4	0.356	-0.016	-0.060	0.027
R / B	-2.826	4	**0.048**	-0.032	-0.063	-0.001
R / T	-3.315	4	**0.030**	-0.029	-0.053	-0.005
B / T	-1.145	4	0.316	-0.016	-0.056	0.023
R / B / T	-1.491	4	0.210	-0.046	-0.131	0.039

	Total cells Test Value = 0					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
R	-3.108	4	**0.036**	-0.040	-0.076	-0.004
B	-0.275	4	0.797	-0.003	-0.035	0.028
T	-1.714	4	0.162	-0.024	-0.063	0.015
R / B	-0.548	4	0.613	-0.005	-0.028	0.019
R / T	-1.593	4	0.186	-0.012	-0.032	0.009
B / T	-0.225	4	0.833	-0.003	-0.038	0.032
R / B / T	-0.195	4	0.855	-0.002	-0.035	0.030

Table 5. 5 P value and correlation coefficient of per capita interactions with *G. incrustans* and total diatom cells.

	Dynamic Index	
	AFDM	**Chlorophyll a**
G. incrustans		
Pearson Correlation	0.033	0.087
P (2-tailed)	0.852	0.619
Total diatoms		
Pearson Correlation	0.501	0.459
P (2-tailed)	0.002	0.006

Table 5. 6 Results of paired t-tests (t statistic and p value) comparing observed and expected values of the dynamic index (AFDM, chlorophyll *a*, total cell count) for snail combination treatments.

	AFDM		**Chlorophyll *a***		**Total cell count**	
	t	**p**	**t**	**p**	**t**	**p**
R. balthica / B. tentaculata	-0.68	0.536	-0.67	0.542	-1.68	0.169
R. balthica / T. fluviatilis	-1.28	0.269	-1.47	0.215	-2.52	0.065
B. tentaculata / T. fluviatilis	0.05	0.965	0.05	0.966	-1.16	0.311
All 3 snail species	-0.28	0.795	-0.37	0.731	-0.28	0.795

Figure 5. 1 Mean ± 1 SE per capita impacts of snail grazers on AFDM, Chlorophyll *a* and total algal cell count.

Treatments are: **R** = *Radix balthica*; **B** = *Bithynia tentaculata*; **T** = *Theodoxus fluviatilis*; **R/B** = *R. balthica* and *B. tentaculata*; **R/T =** *R. balthica* and *T. fluviatilis*; **B/T** = *B. tentaculata* and *T. fluviatilis*; and **R/B/T** = all three snail species.

Impacts significantly different from zero are denoted by ***.**

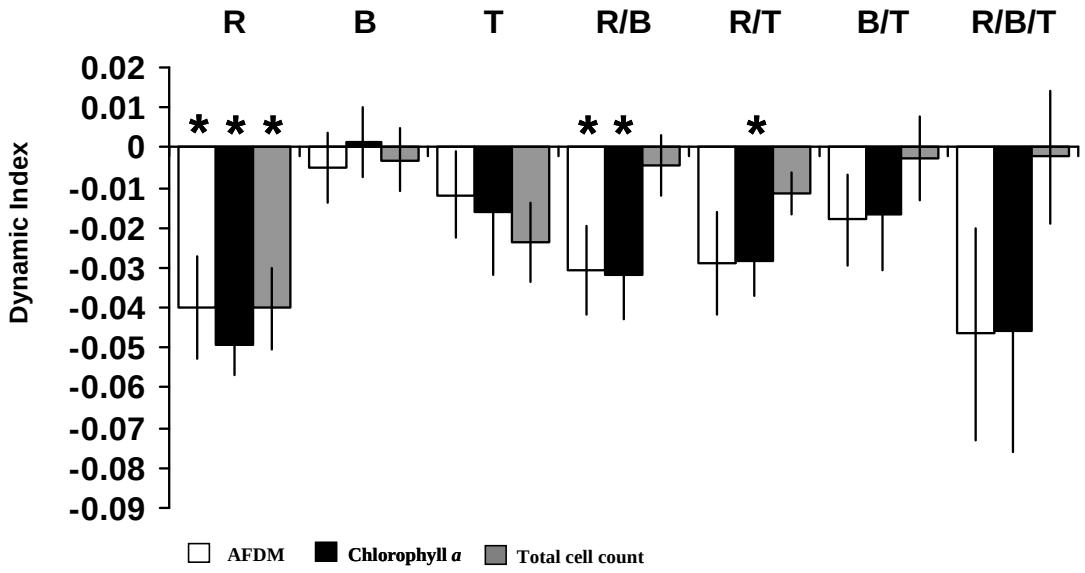


Figure 5. 2 Mean ± 1 SE cell counts per tile for dominant algal groups, a) *G. incrustans*, b) total diatoms and c) *Pleurocapsa* sp.

Treatments are: **R** = Radix balthica; **B** = Bithynia tentaculata; **T** = Theodoxus fluviatilis; **R/B** = R. balthica and B. tentaculata; **R/T** = R. balthica and T. fluviatilis; **B/T** = B. tentaculata and T. fluviatilis; **R/B/T** = all three species. Differences between means are denoted by different letters.

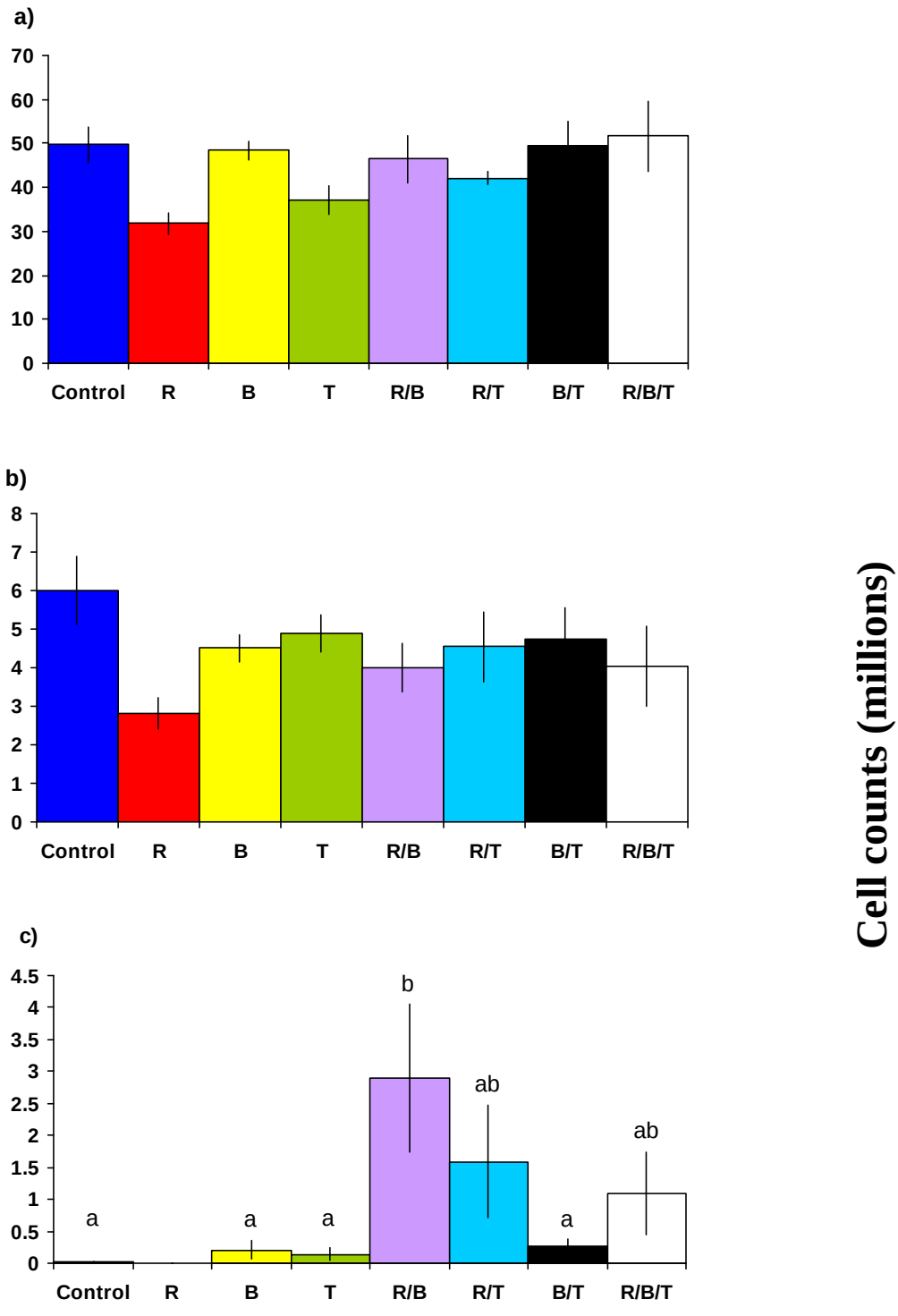


Figure 5. 3 Cell counts for the most abundant diatom species. Treatment means + / - 1 SE are plotted.

Treatments are: **R** = Radix balthica; **B** = Bithynia tentaculata; **T** = Theodoxus fluviatilis; **R/B** = R. balthica and B. tentaculata; **R/T** = R. balthica and T. fluviatilis; **B/T** = B. tentaculata and T. fluviatilis; **R/B/T** = all three species. Differences between means are denoted by the coloured *

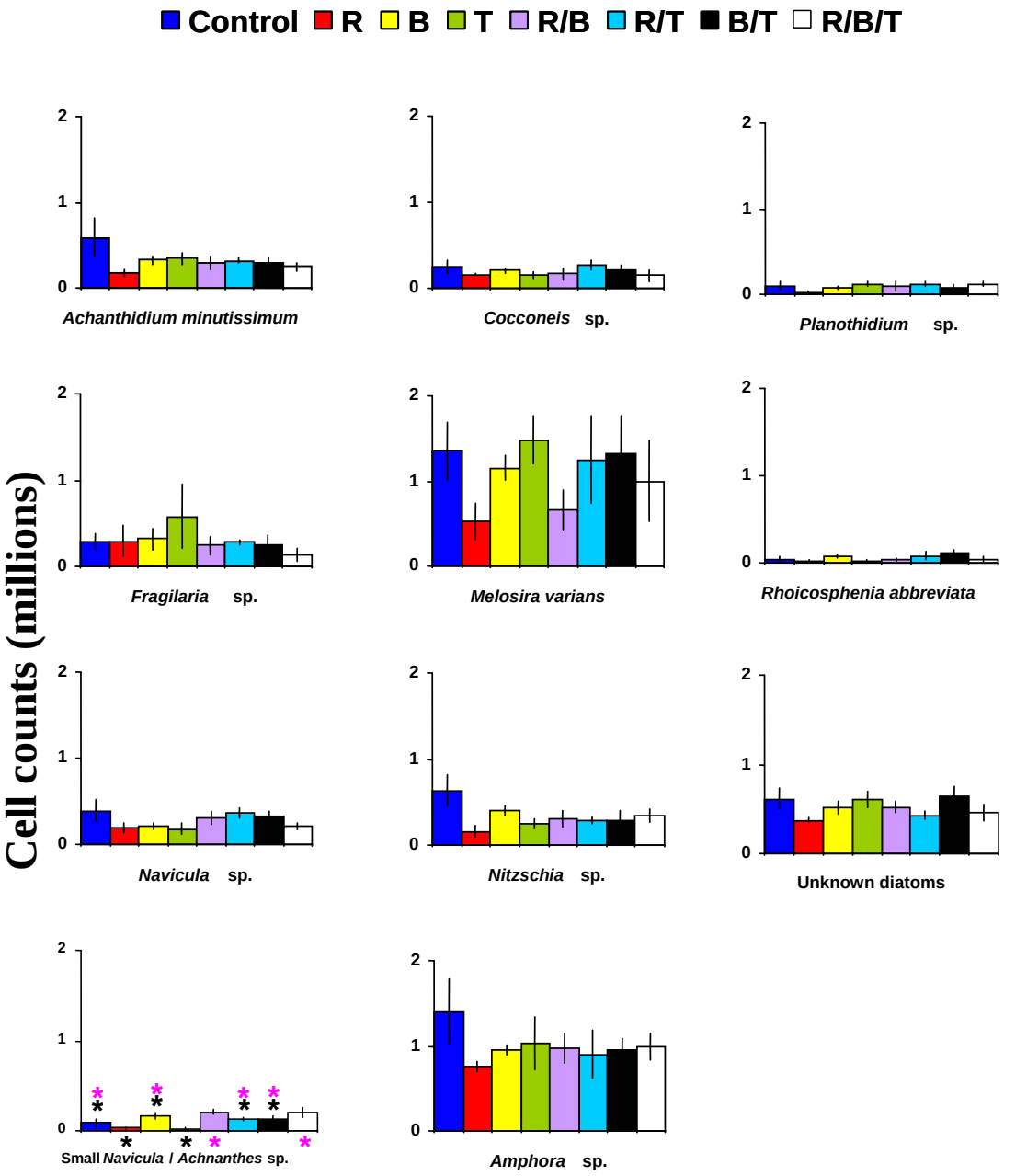


Figure 5. 4 Relationship between the proportion of a) *G. incrustans* and b) total diatoms with per capita snail impact across all treatments.

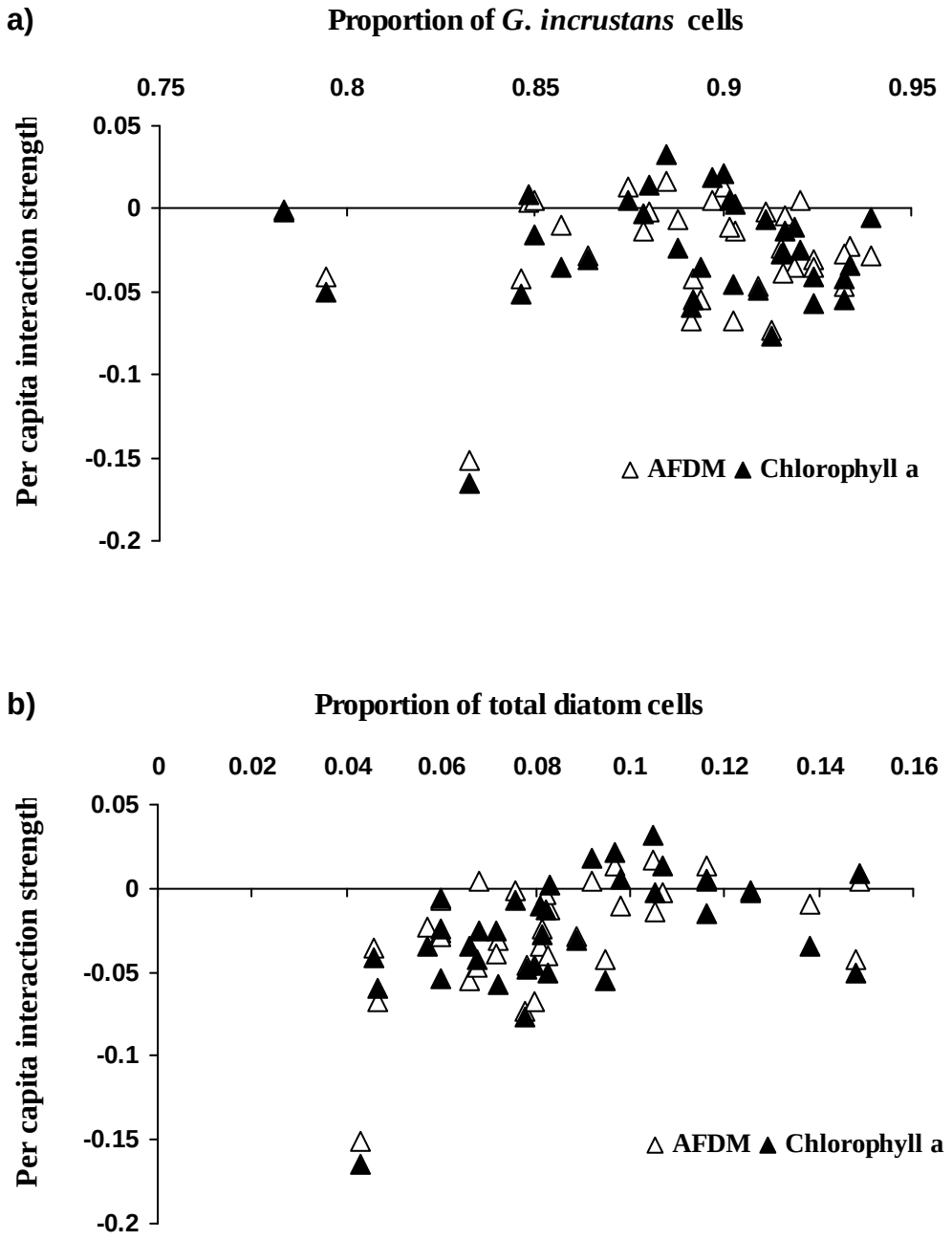


Figure 5. 5 Mean ± 1 SE per capita interactions (dynamic index AFDM) for *G. incrustans* and total diatom cell abundance across snail treatments.

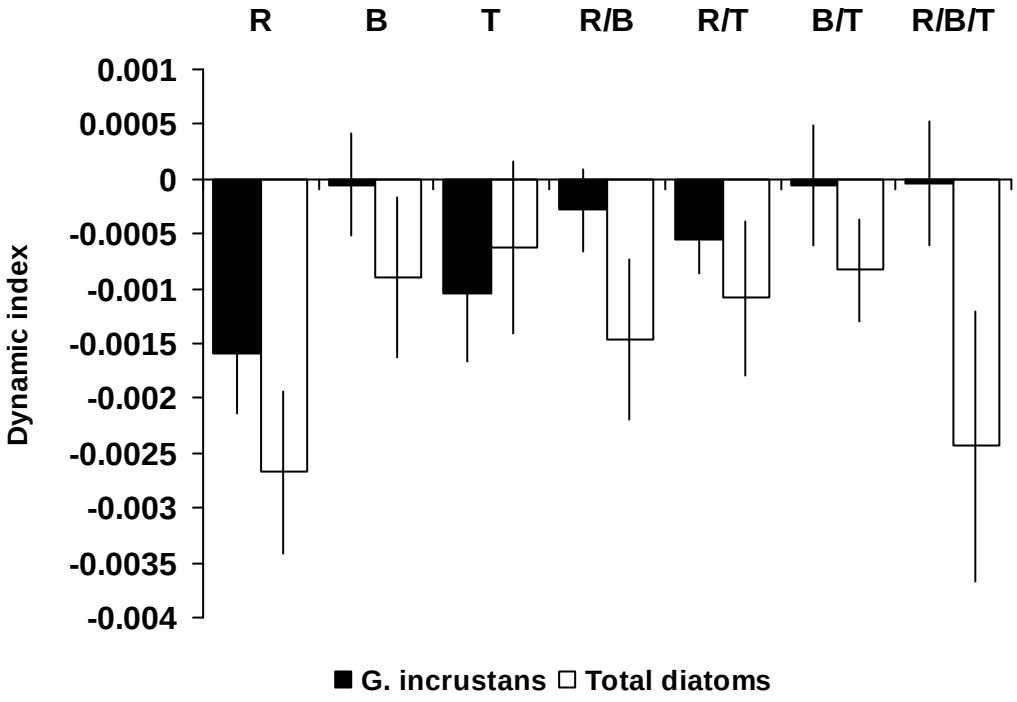
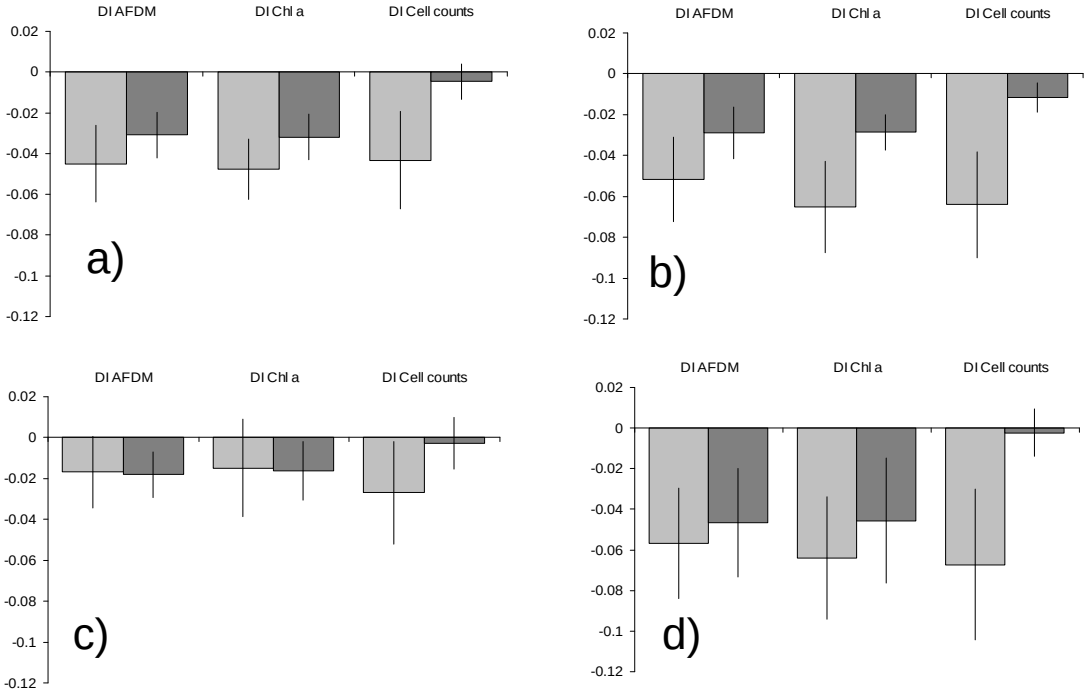


Figure 5. 6 Mean ± 1 SE observed (dark grey bars) versus expected (pale grey bars) values of the dynamic index (AFDM, chlorophyll *a*, total cell count) for snail species combination treatments:

a) *R. balthica* / *B. tentaculata* b) *R. balthica* / *T. fluviatilis* c) *B. tentaculata* / *T. fluviatilis* d) all three snails.



CHAPTER 6 – GENERAL DISCUSSION

6.1 Interaction strength distributions and predicting strong interactions

Grazer manipulation experiments in all chapters showed consistent topographical patterns, dominated by many weak links between herbivores and the algal biofilm, regardless of biofilm type or invertebrate species. Theoretical and empirical approaches consistently find this type of structural arrangement across ecosystem types (Paine, 1992; Wootton, 1997; Laska and Wootton, 1998; Abrams 2001; Sala and Graham, 2002; Berlow et al., 1999, 2004). Many weak interactions may, therefore underpin a stable arrangement of consumer links in this chalk stream system, which improves resistance to ecological change. Conversely, strong food web links appeared to be unstable and vulnerable to specific contextual limitations, such as the presence of *Gongrosira incrustans*, grazer identity, body size, competition and combinations of these factors. Recent research geared towards identifying where strong consumer links occur in complex connectence webs suggests body size as a potential proxy for interaction strength (Sala and Graham, 2002; Woodward and Hildrew, 2002; Emmerson and Raffaelli, 2004). Body size is an important function of energy budgets and consumer metabolic requirements, particularly at high trophic levels where predators and prey are closely matched in size (Cohen et al., 1993). Large predators can consume a wide range of prey items, whereas smaller predators are gape limited (Woodward and Hildrew, 2002). Body size distributions among freshwater algal-herbivore interactions may not however, be a panacea for predicting interaction magnitude, because algal prey items are an order of magnitude smaller than

herbivorous macro-invertebrate consumers. Thus, larger sized grazers have only negligible trophic advantages over smaller consumers. However, a large bulky body can displace significant algal biomass as a grazer forages (Steinman, 1996). This thesis demonstrates that a large size and grazer identity equally shape interaction strength, for example larger snail grazers did induced greater shifts in algal biomass, but the precise identity of the strong link fluctuated between snail species according to biofilm characteristics.

Initial algal biomass (particularly Chlorophyll *a*) and diatom abundance (figure 6.1 a-d) was lower in chapter 2. The biofilm was visibly thinner than chapters 3, 4 and 5, which were all high in diatoms; except for crust biofilms in chapter 3 (e.g. figure 6.2 which compare chapter 2 biofilms and mat assemblages from chapter 3). Thickness of the biofilm therefore had implications for exploitation by snails. *Theodoxus fluviatilis* was more effective than *Radix balthica* at depleting a thin biofilm (figure 6.2 a and b respectively), yet on a thicker diatom mat *T. fluviatilis* was relatively poor compared to *R. balthica* (figure 6.2 e and d respectively). Interestingly *R. balthica* appeared to reduce mat biofilms to a similar level regardless of initial algal biomass (figure 6.2 b and d).

A recent review (see Berlow et al., 2004) extricating the complex topic of interaction strength within ecological networks advocates empirically derived estimates to determine link magnitude, including body size and other traits such as foraging modes and biomass distribution. The application of biological proxy's to determine strong interaction coefficients are perhaps more appropriate at higher trophic levels, while a multi-proxy approach may elucidate a more precise measure of interaction strength at lower levels.

Nevertheless, decoding the identity of strong links for predicting web stability may not be important in productive systems like the Mill Stream, where multiple weak interactions act to dampen the effects of strong grazers. McCann et al. (1998) suggest a contrast in web stability exists between productive systems dominated by generalist consumers (stable), and depauperate webs dominated by specialist consumers with an inherently strong average interaction matrix (less stable). Under this scenario it is clear that productivity has an important role in determining interaction strength distributions. Perhaps, overall productivity within a system would better explain interaction distributions, rather than isolating individual predator prey relationships (e.g. Paine, 1992; Jonsson and Ebenman, 1998; Sala and Graham, 2002). Such an approach would also incorporate non-trophic interactions such as competition, facilitation and disturbance, all of which were flagged by Berlow et al., (2004) as important ecological components that contribute to web dynamics. Furthermore, methods that avoid using calculated metrics, such as the dynamic index, which has inherent biases and errors associated with it (Laska and Wootton, 1998; Berlow et al., 1999), lessens our dependence on controlled experimental model systems (see chapter 2 for a critique of mesocosms) for calculating such metrics.

6.2 Interaction strength – range of effects

The widest range of grazer impacts (strong positive to strong negative dynamic index values) were detected across a diverse herbivore guild (figure 6.3, chapter 2). Positive algal growth was strongest for non-grazing herbivores. Intermediate or low level consumption by herbivores can profit biofilms, by two main mechanisms; 1) poor grazing ability and 2) grazer induced nutrient supply to algal cells. Nutrient recycling;

gardening of algal cells; mis-match between consumer feeding morphology and algal physiognomy etc all contribute to the mechanisms above, see Feminella and Hawkins (1995) and Steinman (1996) for comprehensive reviews of these processes. Less consistent positive effects in chapters 3, 4 and 5 are congruent with specific selection of predominantly grazing target species exerting weaker positive effects. The greater range of negative interactions in chapter 2 compared to other chapters was dominated by the extreme effect of *T. fluviatilis*, which suggests it is more effective on thinner algal mats over relatively short time (0.96 days). Hillebrand et al (2004) also found a very strong effect of *T. fluviatilis* on algal biomass, significantly depressing biomass after 2 days of experimentation. However, this was the only experiment where *T. fluviatilis* had a disproportionately large impact. Furthermore, in chapters 3 and 5 this snail exhibited a 'digger' foraging mode (Wilson et al, 1999; Chase et al, 2001) that exerted a greater impact on the *Gongrosira incrustans* element of crust biofilms. *Theodoxus fluviatilis* effectively foraged on a sparse biofilm in chapter 2, which suggests it intensely removed algae as it moved over the mat (figure 6.2 a), conversely *R. balthica* showed evidence of a less intensive 'grazer' foraging strategy (Wilson et al., 1999; Chase et al., 2001), which left remnant algae free to reproduce (figures 6.2 b and d). Therefore, thick diatom mats (chapter 3) appear to be impervious to diggers (*T. fluviatilis* and *Bithynia tentaculata*), but less resistant to grazers.

The range in grazer impacts (DI) for Chapters 3 and 5 were narrow (figure 6.3), but each had comparatively more *G. incrustans* integrated into biofilms (figure 6.1), suggesting grazer effects were weak on this algae. However, *G. incrustans* is common and wide-spread in calcareous systems (Butcher, 1946) is the dominant green alga in the River Frome and Mill Stream (Marker and Casey, 1982; Ledger et al., 2008); and

persists during the winter (Pentecost, 1988). Current knowledge of this dominant green alga in relation to grazing pressure is poor.

In summary, the level to which interactions are altered for individual grazer species is highly dependent upon spatial and temporal context. For example the three main snail species used in this project (*R. balthica*, *T. fluviatilis* and *B. tentaculata*) all exhibited variable impacts on biofilms in different chapters, where grazer and algal attributes were experimentally controlled. Natural systems that are extremely variable both spatially and temporally may therefore intensify context-dependent interaction strengths. Furthermore, depending on when and where experiments are conducted the typical 'snap-shot' of community function and / or distribution of link strengths may not be accurate at assessing the same suit of organisms over space and time.

6.3 Pertinent effects of snails

Overall impacts of snails were stronger than impacts of mayflies in all chapters. Due to differences in feeding mode, mouthpart morphology and mode of locomotion (see Sumner and McIntire, 1982; Hill and Knight, 1988; Lamberti et al., 1989; McCormick, 1994; Allan, 1995; Steinman, 1996; Fuller and Desmond, 1997; Wilson et al., 1999 for examples). Furthermore, snails are numerically abundant and diverse in the Mill Stream, occupying 22-67% of total fauna in gravel areas free from macrophyte cover (Welton et al., 1983). Consequently, in this system snails are paramount in converting algal biomass into secondary production. One major route of energy transfer from herbivores to tertiary consumers occurs through predation (Giller and Malmqvist, 1998), i.e. fish predation and to a lesser extent invertebrate predation. However, gastropod snails, although a component of fish diet, are not as frequently

consumed as insect prey (Mann and Orr, 1969; Elliot, 1973; Welton et al, 1983). Fish predation of snails is limited because; large specimens can reach a size refugium, the presence of the snail shell reduces palatability; and locomotion, as fish preferentially exploit more motile species such as insect larvae (Welton et al., 1983). This poses the question of how energy from algae is transmitted to higher consumers via snails. Viable routes of energy transfer from snails to other members of the food chain could occur through faecal deposition, production of egg masses and decomposition of dead snails. Wotton and Malmqvist (2001) highlight the importance of faeces as a food resource for deposit and filter feeders, and also mayflies which will transfer energy further up. Brendelberger (1997), on the other hand shows the importance of faecal recycling for snail growth in less productive systems, where secondary consumption of some food resources enhances digestibility. Little information is available on snail egg mass production, however large quantities of snail egg masses were observed during this study (see figure 6.2 d – showing a large *R. balthica* egg mass). There are no studies that specifically look at freshwater snail egg masses as a food source. Eggs surrounded by a tough gelatinous casing appeared to be tightly attached to the substratum (Personal observation). Consequently, these egg masses may not be easily utilised by potential consumers. Further research into the importance of snail egg masses may therefore be of interest. The suggested routes of energy transfer are most relevant to systems where snails are the dominant grazing animals. However, where snails are less abundant (e.g. calcium poor systems) energy transfer may thus follow more conventional routes from algae to insect larvae, and then to secondary and tertiary consumers.

6.4 Consumer effects

Algal response variables measured in this thesis combined consumptive and export loss for each grazer across different biofilms (chapters 2, 3, 4 and 5). Measuring these overall effects incorporates trophic interactions with export, which is valuable for estimating true algal-herbivore dynamics within patches. Lamberti et al. (1987) found that algae lost via mechanical dislodgement substantially contributed to grazer impacts, with larger, less agile grazers like snails and cased caddis exporting more loosely attached overstory layers than smaller less cumbersome mayfly nymphs. Other studies have reported similar effects of grazers on algal export (Hill and Knight, 1988; Lamberti et al., 1989; Barnese et al., 1990; DeNicola and McIntire, 1991; McCormick, 1994). Consequently, grazing within small algal patches may be offset over larger spatial scales with export of algal cells promoting re-colonisation and may also facilitate capture by other FFG's such as deposit and filter-feeding animals (Lamberti et al., 1987). Moreover, examination of biofilms after grazer removal provides information about which algae are resistant to grazing pressure. Remnant algal species resist grazing by strong attachment close to the substrate, such as small adnate species like *Achnanthidium minutissimum* and *Cocconeis* spp. (Chapter 2; Lamberti et al., 1987; Peterson, 1987; Barnese and Lowe, 1992). Larger overstory species on the other hand, tend to incur greater impacts of grazing invertebrates (Chapter 3; Sumner and McIntire, 1982; Underwood et al., 1992). However, using the overall consumptive effect of grazers makes it difficult to assess algal resilience. Grazing resilience may be associated with increased productivity of smaller algae

with short generation times when stimulated by grazing (Steinman, 1996). Under intermediate grazing pressure these algae rapidly reproduce as light and nutrients become more available through the removal of overstory species (Lamberti and Resh, 1983; Liess and Hillebrand, 2004). Consequently, grazer induced cell loss may be counterbalanced by higher productivity, and result in weak net effects of grazing. Therefore, estimation of grazer impacts on some algal taxa cannot be inferred from analysing remnant algal species alone, because proportions of algal taxa in grazed and ungrazed treatments may be equivalent. Comparing the proportions of algal taxa in remnant biofilms to their proportions in grazer guts can help clarify this. For example, a grazer which has higher proportions of *A. minutissimum* in its gut compared to the grazed biofilm suggests that this diatom is readily available but not highly represented among remnant cells i.e., products of high turn over are quickly ingested by grazers.

Analysis of herbivore gut contents also separates trophic impacts from non-trophic effects, such as mechanical export. Gut contents were not formally analysed for this thesis due to time limitation, but preliminary work suggests that diatoms were the dominant cells present in herbivore guts (figure 6.4). However, a comprehensive analysis of herbivore guts from chapter 2 might also prove useful in comparing the relative proportions of algae, detritus and POM in grazing versus non-grazing herbivores. This kind of analysis provides information on resource partitioning i.e., how non-grazers may supplement their diet with herbivory.

Gut contents analysis is widely used to identify trophic links between organisms in connectence webs, by directly observing consumed items. Although this method is probably the best way of assessing who eats whom, some issues of accuracy exist when examining herbivore guts. Algal cells are differentially digested depending on the grazer and algal species consumed. The siliceous frustules of diatoms are

relatively indestructible (standard diatom preparation involves acid oxidation to remove organic matter, allowing frustule surface features used in identification to stand out). With this level of protection, gut analysis is biased towards diatom enumeration. Furthermore, it is difficult to determine if diatoms were alive or dead when consumed unless viable chloroplasts are still visible inside the cell. This is where grazer identity can affect the likelihood of observing chloroplasts. Peterson et al (1998) found that mayfly nymphs (*Ameletus* sp. [Ameletidae]) were more efficient at digesting diatom cells than a caddis competitor (*Ecclisomyia* sp. [Limnephilidae]). Similarly Diaz Villanueva and Albarino (2003) observed that diatoms were more efficiently digested by *Meridialaris diguillina* (Leptophlebiidae) than *Baetis* sp. (Baetidae). Therefore depending on the level of digestion and gut retention time, biased estimates may arise. Adding to this, green (Chlorophyta) and blue / green algae (Cyanophyta) are generally more easily digested and can be unrecognisable in gut contents, which can cause an underestimate of these cells in dietary analysis (Peterson et al., 1998). Consequently a combined approach comparing remnant biofilm composition and gut contents analysis will help disentangle trophic links from effects of transport.

6.5 Mesocosms

It is important to stress that mesocosms do not represent natural systems and the complex interactions that occur within them (Abrams, 2001). They are merely an instrument ecologists use to examine species interactions, and caution must be used when extrapolating mesocosm results to the real world (Steinman, 1991). However, the frequent use of mesocosms or laboratory streams and bank side channels in

ecology re-enforces their use as a practical tool to interpret interactions in model stream systems (e.g. Jacoby, 1985; Steinman, 1991; Tuchman and Stevenson, 1991; Rosemond et al., 1993; Brown et al., 1994; Gresens and Lowe, 1994; McCormick, 1994; Kawata et al., 2001; King-Lotufo et al., 2002, Ledger et al., 2006, 2008).

Field based mesocosm experiments were used to maintain physiochemical conditions as close to those in the Mill Stream as possible, such as light, nutrients, temperature, pH and conductivity. The use of mesocosms gave greater control over the algae and grazers under manipulation compared to benthic enclosures or exclosures, where immigration from the benthos can be problematic (Lamberti and Feminella, 1996) or loss of experimental units due to spates is possible. All experimental algae and grazers were collected from the Mill Stream itself or the River Frome, thus ensuring their co-occurrence in the natural stream. Algae and invertebrates used in experiments reflected possible combinations of the natural Mill Stream community (Chapter 2). Although algal biomass was higher in the mat biofilm of chapter 3, this was typical of diatom filaments containing *Melosira varians*, abundant in the Mill Stream channel during the summers of 2005 and 2006 (personal observation). This particular form of biofilm is difficult to sample because of its flocculent nature and loose attachment (personal observation; Ledger et al., 2008). Therefore stone scrapes taken during the survey may not have accurately represented this particular biofilm physiognomy.

Experiments using mesocosms have particular problems associated with them: i.e. confined space, restricted flow and edge effects, although the latter is more prominently associated with predator-prey enclosures where encounter rates are high. Algal prey species are relatively sedentary compared to grazers and are exploitable by grazers within small patches regardless of mesocosm edges. Using a short experimental period (3 days) alleviates edge effects by restricting the length of time

algae and grazers are exposed to potential cage effects (Brown et al., 1994; King-Lotufo et al., 2002). In separate studies, both King-Lotufo et al (2002) and Hillebrand et al (2004) found strong snail effects in short duration experiments investigating snail impacts on algae.

6.6 Further work

Effects of predators

A logical step from this research would be to evaluate the impact of predators on grazing. Extensive research has been conducted on trophic cascades across many different systems (Pace et al., 1999; Knight et al., 2005; Shurin et al., 2002). Fish can have significant indirect effects on algal biomass by directly consuming invertebrate grazers (Power, 1990; Bechara et al., 1992, 1993; Harvey, 1993; Rosenfeld, 2000). However, the effect of trophic cascades has not been investigated in this chalk stream environment where snail grazers dominate algal biomass depression. Snails are less often consumed by fish predators (Welton et al., 1983), so what are the effects of fish in regulating algal biomass through cascades in the Mill Stream? Conversely, mayflies are more susceptible to fish predation (Welton et al., 1983), but exert weaker impacts on biofilms over short time scales at least. Thus, would cascades be more likely to occur through mayfly predation? Investigating the effects of predation on grazers would reveal the transfer of energy from primary consumers to tertiary consumers in this complex chalk stream system. Experiments designed to investigate how grazers react to fish cues i.e. the actual presence of fish predators or chemical fish cues, could be used to test the effects of fish that feed in the benthos, e.g.

bullhead or the water column, e.g. salmonids. This can be investigated using enclosures and enclosures (of fish and grazers) in the Mill Stream benthos, in laboratory studies as stream experimentation offers more realism but is less controllable.

Effects of season

Gongrosira incrustans persists into winter while diatoms are less abundant (Pentecost, 1988). Therefore, how does the grazing community differ with lower diatom resources? Furthermore macrophytes are not an important factor in winter, thus biofilm inputs to overall stream production may be proportionally more important during the winter months. Insects can over-winter as larvae, but most are small and usually only large species such as the detritivore mayfly *Ephemera* sp. (Ephemeridae) are large during winter (Elliott et al., 1988). However, snail life-cycles permit the overlap of cohorts during the winter. Therefore, how do snails interact with winter biofilms?

6.7 Summary

The distribution of interaction strength was biased towards many weak links and fewer strong links, mainly due to the impact of two snail species (research aim 1). Interaction strength was context dependent and varied between crust and mat patch types, where it was generally higher (research aim 2). Interaction strength increased with grazer body size, though this was only evident for the larger snails (research aim

3). Interaction strength appeared to be weaker when several species of snail were combined, though this effect was marginal (research aim 4).

6.8 References

Abrams, P. A. (2001) Describing and quantifying interspecific interactions: a commentary on recent approaches. **Oikos**, 94: 209-218.

Allan, J. D. (1995) **Stream ecology: Structure and function of running waters.** London: Chapman and Hall.

Barnese, L. E. and Lowe, R. L. (1992) Effects of Substrate, Light, and Benthic Invertebrates on Algal Drift in Small Streams. **Journal of the North American Benthological Society**, 11(1): 49-59.

Barnese, L. E., Lowe, R. L. and Hunter, R. D. (1990) Comparative grazing efficiency of pulmonate and prosobranch snails. **Journal of the North American Benthological Society**, 9(1): 35-44.

Bechara, J. A., Moreau, G. and Hare, L. (1993) The impact of brook trout (Salvelinus fontinalis) on an experimental stream community. **Journal of Animal Ecology**, 62: 451-464.

Bechara, J. A., Moreau, G. and Planas, D. (1992) Top down effects of brook trout (Salvelinus fontinalis) in a boreal forest stream. **Canadian Journal of Fisheries and Aquatic Sciences**, 49: 2093-2103.

Berlow, E. L. (1999) Strong effects of weak interactions in ecological communities. **Nature**, 398(6725): 330-334.

Berlow, E. L., Neutel, A. M., Cohen, J. E., et al. (2004) Interaction strengths in food webs: issues and opportunities. **Journal of Animal Ecology**, 73(3): 585-598.

Brendelberger, H. (1997) Coprophagy: a supplementary food source for two freshwater gastropods? **Freshwater Biology**, 38: 145-157.

Brown, K. M., Carman, K. R. and Inchausty, V. (1994) Density-dependent influences on feeding metabolism in a freshwater snail. **Oecologia**, 99: 158-165.

Butcher, R. W. (1946) Studies in the ecology of Rivers: VI. The algal growth in certain highly calcareous streams. **Journal of ecology**, 33(2): 268-283.

Chase, J. M., Wilson, W. G. and Richards, S. A. (2001) Foraging trade-offs and resource patchiness: theory and experiments with a freshwater snail community. **Ecology Letters**, 4: 304-312.

Cohen, J. E., Pimm, S. L., Yodzis, P., et al. (1993) Body sizes of animal predators and animal prey in food webs. **Journal of Animal Ecology**, 62: 67-78.

DeNicola, D. M. and McIntire, C. D. (1991) Effects of Hydraulic Refuge and Irradiance on Grazer-Periphyton Interactions in Laboratory Streams. **Journal of the North American Benthological Society**, 10(3): 251-262.

Diaz Villanueva, V. and Albarino, R. (2003) Algal ingestion and digestion by two Ephemeropteran larvae from a Patagonian Andean stream. **Research update on Ephemeroptera and Plecoptera**: 469-475.

Elliott, J. M. (1973) The food of brown and rainbow trout (Salmo trutta and S. gairdneri) in relation to the abundance of drifting invertebrates in a mountain stream. **Oecologia**, 12: 329-347.

Elliott, J. M., Humpesch, U. H. and Macan, T. T. (1988) **Larvae of the British Ephemeroptera: a key with ecological notes. Scientific Publication No. 49.** Ambleside: Freshwater Biological Association.

Emmerson, M. C. and Raffaelli, D. (2004) Predator-prey body size, interaction strength and the stability of a real food web. **Journal of Animal Ecology**, 73: 399-409.

Feminella, J. W. and Hawkins, C. P. (1995) Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. **Journal of the North American Benthological Society**, 14(4): 465-509.

Fuller, R. L. and Desmond, C. (1997) Influence of food type on the growth of early and late instars of three mayfly (Ephemeroptera) species. **Archiv Fur Hydrobiologie**, 141(1): 1-11.

Giller, P. S. and Malmqvist, B. (1998) **The biology of streams and rivers.** Oxford: Oxford university press.

Gresens, S. E. and Lowe, R. L. (1994) Periphyton Patch Preference in Grazing Chironomid Larvae. **Journal of the North American Benthological Society**, 13(1): 89-99.

Harvey, B. C. (1993) Benthic assemblages in Utah headwater streams with and without trout. **Canadian Journal of Zoology**, 71: 896-900.

Hill, W. R. and Knight, A. W. (1988) Concurrent Grazing Effects of 2 Stream Insects on Periphyton. **Limnology and Oceanography**, 33(1): 15-26.

Hillebrand, H., de Montpellier, G. and Liess, A. (2004) Effects of macrograzers and light on periphyton stoichiometry. **Oikos**, 106: 93-104.

Jacoby, J. M. (1985) Grazing effects of periphyton by Theodoxus fluviatilis (Gastropoda) in a lowland stream. **Journal of Freshwater Ecology**, 3(2): 265-274.

Jonsson, T. and Ebenman, B. (1998) Effects of predator-prey body size ratios on the stability of food chains. **Journal of Theoretical Biology**, 193(3): 407-417.

Kawata, M., Hayashi, M. and Hara, T. (2001) Interactions between neighbouring algae and snail grazing in structuring microdistribution patterns of periphyton. **Oikos**, 92: 404-416.

King-Lotufo, E. C., Brown, K. M. and Carman, K. R. (2002) The influence of periphyton biomass and density on grazing in Physella virgata. **Hydrobiologia**, 482(1-3): 23-29.

Knight, T. M., McCoy, M. W., Chase, J. M., et al. (2005) Trophic cascades across ecosystems. **Nature**, 437(7060): 880-883.

Lamberti, G. A., Ashkenas, L. R., Gregory, S. V., et al. (1987) Effects of three herbivores on periphyton communities in laboratory streams. **Journal of the North American Benthological Society**, 6(2): 92-104.

Lamberti, G. A. and Feminella, J. W. (1996) "Plant-Herbivore interactions". In Hauer, F. R. and Lamberti, G. A. (ed.) **Methods in stream ecology.** London, Academic press. pp. 409-430.

Lamberti, G. A., Gregory, S. V., Ashkenas, L. R., et al. (1989) Productive Capacity of Periphyton as a Determinant of Plant Herbivore Interactions in Streams. **Ecology**, 70(6): 1840-1856.

Lamberti, G. A. and Resh, V. H. (1983) Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. **Ecology**, 64(5): 1124-1135.

Laska, M. S. and Wootton, J. T. (1998) Theoretical concepts and empirical approaches to measuring interaction strength. **Ecology**, 79(2): 461-476.

Ledger, M. E., Harris, R. M. L., Armitage, P. D., et al. (2008) Disturbance, patchiness and coexistence in freshwater algal communities. **Oecologia**, 155: 809-819.

Ledger, M. E., Harris, R. M. L., Milner, A. M., et al. (2006) Disturbance, biological legacies and community development in stream mesocosms. **Oecologia**, 148: 682-691.

Liess, A. and Hillebrand, H. (2004) Invited review: Direct and indirect effects in herbivore periphyton interactions. **Archiv Fur Hydrobiologie**, 159(4): 433-453.

Mann, R. H. K. and Orr, D. R. O. (1969) A preliminary study of the feeding relationships of fish in a hard-water and a soft-water stream in Southern England. **Journal of Fish Biology**, 1: 31-44.

Marker, A. F. H. and Casey, H. (1982) The population and production dynamics of benthic algae in an artificial recirculating hard-water stream. **Phil. Trans. R. Soc. Lond. B**, 298: 265-308.

McCann, K., Hastings, A. and Huxel, G. R. (1998) Weak trophic interactions and the balance of nature. **Nature**, 395: 794-798.

McCormick, P. V. (1994) Evaluating the Multiple Mechanisms Underlying Herbivore Algal Interactions in Streams. **Hydrobiologia**, 291(1): 47-59.

Pace, M. L., Cole, J. J., Carpenter, S. R., et al. (1999) Trophic cascades revealed in diverse ecosystems. **Trends in Ecology and Evolution**, 14(12): 483-488.

Paine, R. T. (1992) Food-Web Analysis through Field Measurement of Per-Capita Interaction Strength. **Nature**, 355(6355): 73-75.

Pentecost, A. (1988) Observations on growth rates and calcium carbonate deposition in the green alga *Gongrosira*. **New Phytologist**, 110: 249-253.

Peterson, C. G. (1987) Gut Passage And Insect Grazer Selectivity Of Lotic Diatoms. **Freshwater Biology**, 18(3): 455-460.

Peterson, C. G., Vormittag, K. A. and Valett, H. M. (1998) Ingestion and digestion of epilithic algae by larval insects in a heavily grazed montane stream. **Freshwater Biology**, 40(4): 607-623.

Power, M. E. (1990) Effects of fish in river food webs. **Science**, 250(4982): 811-814.

Rosemond, A. D., Mulholland, P. J. and Elwood, J. W. (1993) Top-Down and Bottom-up Control of Stream Periphyton - Effects of Nutrients and Herbivores. **Ecology**, 74(4): 1264-1280.

Rosenfeld, J. S. (2000) Contrasting effects of fish predation in a fishless and fish-bearing stream. **Archiv fur Hydrobiologie**, 147(2): 129-142.

Sala, E. and Graham, M. H. (2002) Community-wide distribution of predator-prey interaction strength in kelp forests. **Proceedings of the National Academy of Sciences of the United States of America**, 99(6): 3678-3683.

Shurin, J. B., Borer, E. T., Seabloom, E. W., et al. (2002) A cross-ecosystem comparison of the strength of trophic cascades. **Ecology Letters**, 5(6): 785-791.

Steinman, A. D. (1991) Effects of herbivore size and hunger level on periphyton communities. **Journal of phycology**, 27: 54-59.

Steinman, A. D. (1996) "Effects of grazers on freshwater benthic algae". In Stevenson, R. J., Bothwell and Lowe, R. L. (ed.) **Algal ecology: Freshwater benthic ecosystems.** London, Academic Press. pp. 341-366.

Sumner, W. T. and McIntire, C. D. (1982) Grazer-periphyton interactions in laboratory streams. **Archiv Fur Hydrobiologie**, 93(2): 135-157.

Tuchman, N. C. and Stevenson, R. J. (1991) Effects of Selective Grazing by Snails on Benthic Algal Succession. **Journal of the North American Benthological Society**, 10(4): 430-443.

Underwood, G. J. C., Thomas, J. D. and Baker, J. H. (1992) An experimental investigation of interactions in snail-macrophyte-epiphyte systems. **Oecologia**, 91: 587-595.

Welton, J. S., Mills, C. A. and Rendle, E. L. (1983) Food and Habitat Partitioning in 2 Small Benthic Fishes, Noemacheilus-Barbatulus (L) and Cottus-Gobio L. **Archiv Fur Hydrobiologie**, 97(4): 434-454.

Wilson, W. G., Osenberg, C. W., Schmitt, R. J., et al. (1999) Complementary foraging behaviors allow coexistence of two consumers. **Ecology**, 80(7): 2358-2372.

Woodward, G. and Hildrew, A. G. (2002) Body-size determinants of niche overlap and intraguild predation within a complex food web. **Journal of Animal Ecology**, 71: 1063-1074.

Wotton, R. S. and Malmqvist, B. (2001) Faeces in aquatic ecosystems. **Bioscience**, 51(7): 537-544.

Wootton, J. T. (1997) Estimates and tests of per capita interaction strength: diet, abundance, and impact of intertidally foraging birds. **Ecological Monographs**, 67(1): 45-64.

6.9 Figures

Figure 6. 1 Mean (+1SE) algal biomass parameters,

a) AFDM, b) Chlorophyll *a*, c) *Gongrosira incrustans* cells, d) diatom cells, and e) *Pleurocapsa* spp. cells, for survey occasions (2005), and experimental biofilms prior to experimentation. Chapter 2 (trials 1 & 2), chapter 3 (mat and crust), chapter 4 (size) and Chapter 5 (snail).

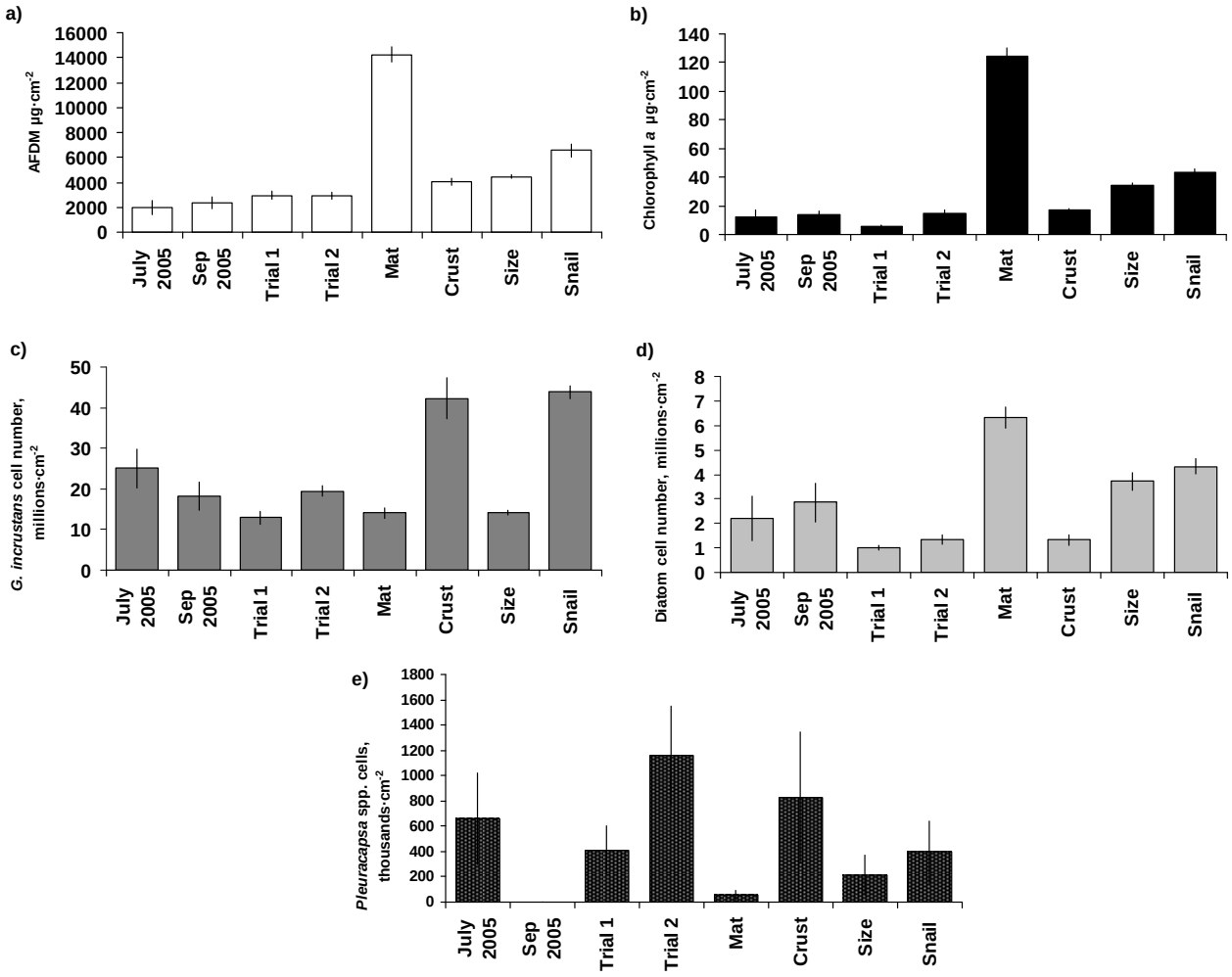


Figure 6. 2 Experimental tiles,

top row: - chapter 2 in-situ tiles [day 1] a) *T. fluviatilis*, b) *R. balthica* and c) control; bottom row: - chapter 3 [mats] tiles removed after termination of experiment; d) *R. balthica*, e) *T. fluviatilis*, f) *B. tentaculata* and g) control

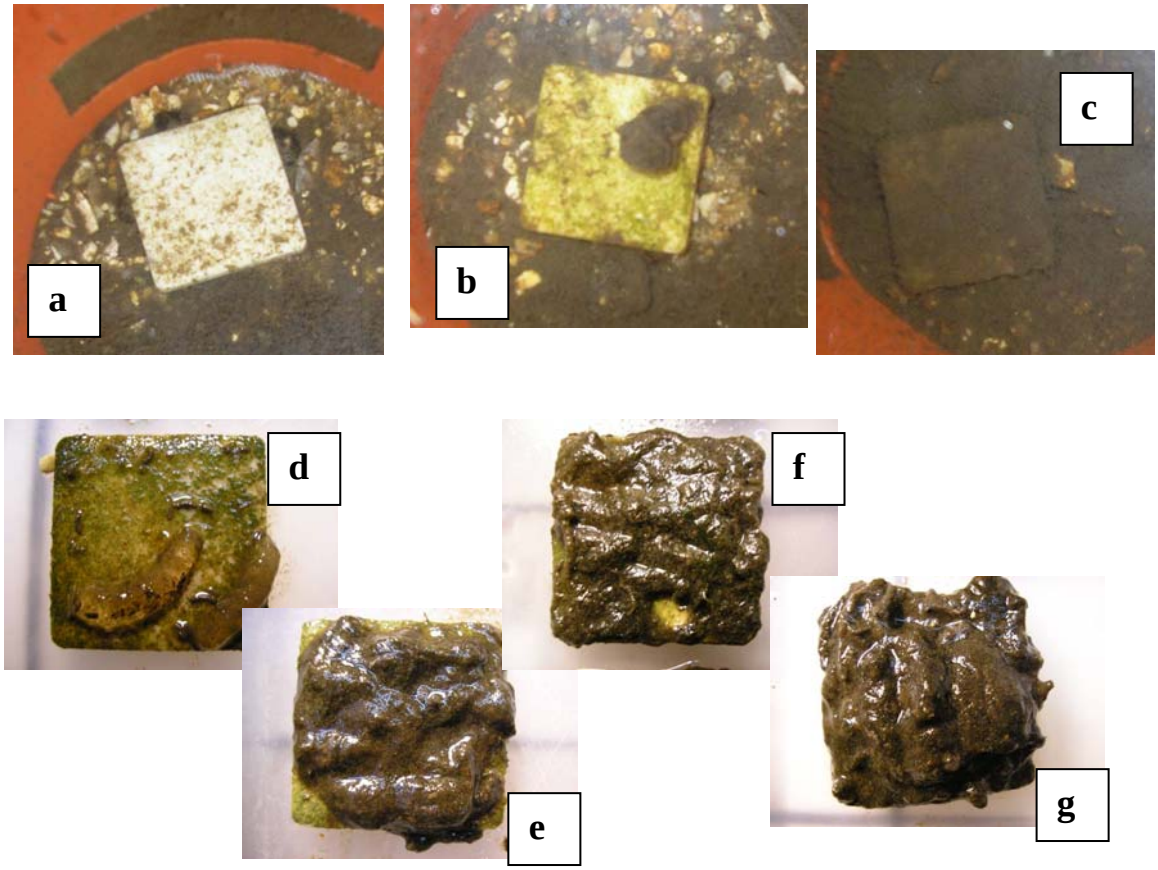


Figure 6. 3 Dynamic index range: Chapters 2, 3 (mat and crust), 4 and 5.
White bar = AFDM; black bars = Chlorophyll *a*; grey bars = total cells.

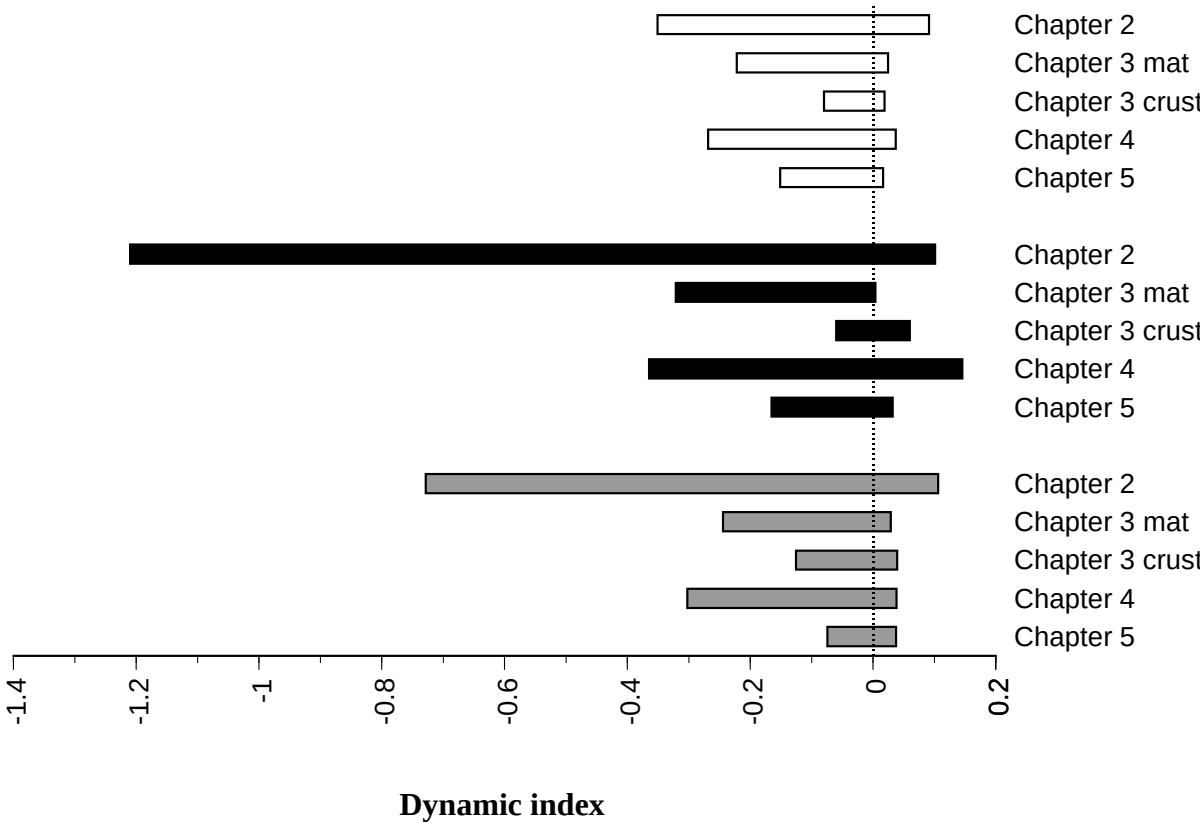
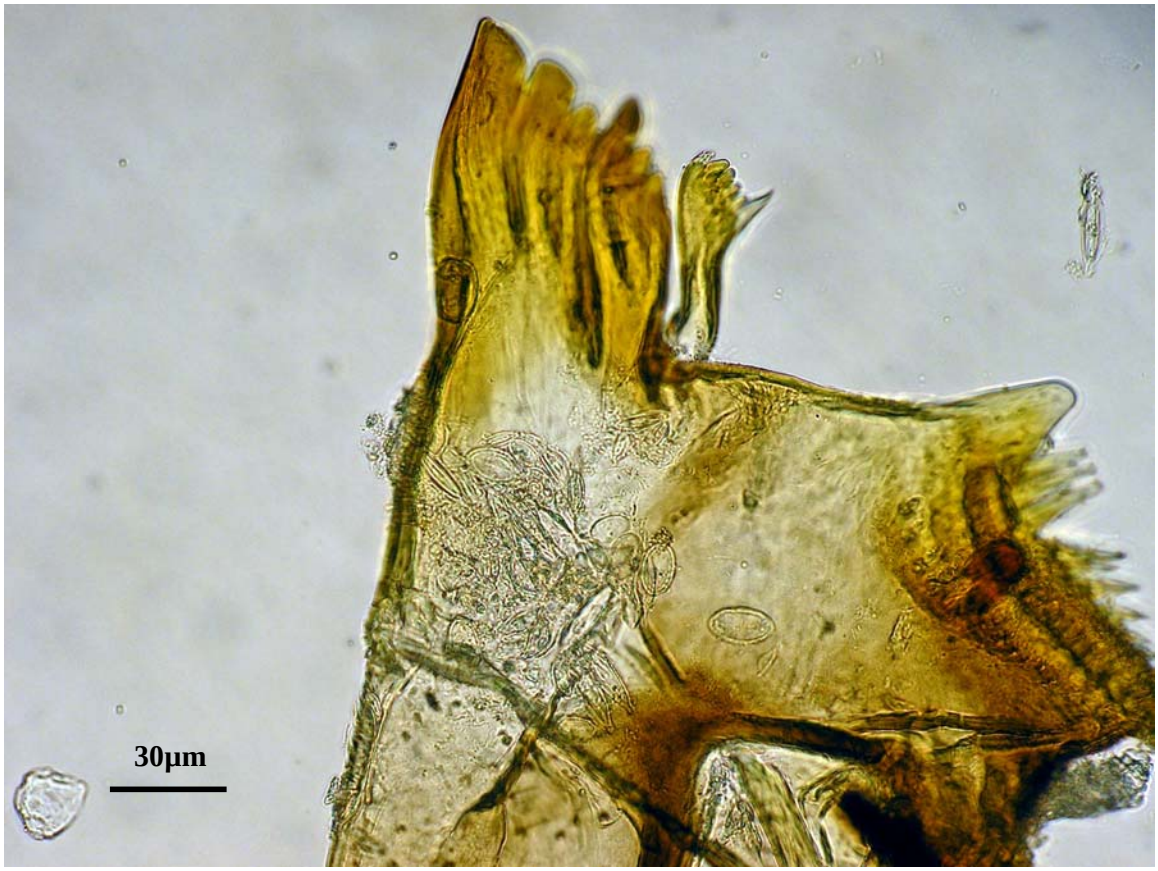


Figure 6. 4 Right mandible of a *B. buceratus* showing harvested diatoms (x 400 magnification).

Cocconeis sp and Naviculoid species are clearly visible.



Appendix 1 – LENGTH-MASS REGRESSIONS

A.1 Introduction

The biomass of organisms is an important parameter in the study of the ecology of natural communities (Woodward et al. 2008) and ecosystems (Steele et al. 2007). For example, estimates of individual biomass are needed to quantify food webs (Hall et al. 2000, Ings et al. 2009), and the strength of the interactions within them (Berlow et al. 2004, Brose et al. 2006). However, it is not possible to weigh every single organism, particularly when the dry mass or ash free dry mass are needed. Biomass can be estimated from length-mass relationships, which must be derived either directly from a sub-sample of individuals, or from published sources (Smock 1980, Meyer 1989, Towers et al. 1994, Burgherr & Meyer 1997, Benke et al. 1999, Cressa 1999, Johnston & Cunjak 1999, Gonzalez et al. 2002, Sabo et al. 2002, Edwards et al. 2008).

There are few published species level length-mass relationships for the snails used in the experiments in chapters 2 to 5 and these often use only one measure of size and weight (Mason 1977, Kirkegaard 2006). The objective of this study was to derive equations predicting dry mass and ash-free dry mass from shell height and width for four snails: *Bithynia tentaculata* L. (Bithyniidae), *Gyraulus albus* Müll. (Planorbidae), *Radix balthica* Müll. (Lymnaeidae) and *Theodoxus fluviatilis* L. (Neritidae).

A.2 Methods

All specimens used in this study were collected from the Mill Stream near East Stoke (see chapter 2) on the same day in July 2005 using kick samples. Samples were fixed in 4 % formaldehyde for two months, then sorted and snail specimens placed in 70 % alcohol for a month. Species identity was confirmed under a microscope (Macan 1994). Specimens of each species, spanning a range of sizes (Table A.1), were measured to the nearest 0.1 mm for shell height (SH) and width (SW) using a microscope with an ocular graticule. The dimensions used as shell height and width for each species were as described by Macan (1994). Specimens were placed in individual pre-weighed foil trays and oven dried at 80 ^{o}C until constant weight, then weighed to nearest 0.01 mg (Sartorius BP121S microbalance) to determine dry mass (DM). The specimens were then ashed at 550 ^{o}C to determine ash free dry mass (AFDM, i.e. DM minus the mass of ash). The least squares regression procedure in Minitab 15 ® was used to derive size-mass equations for the 4 species of snails. Data were Log_{10} transformed and fitted to the linear model:

$$Log_{10}(y) = b \cdot Log_{10}(x) + Log_{10} a$$

Where y, the response variable, was either DM or AFDM in mg; x, the predictor variable, was SH or SW in mm; and a and b were constants. This model is the most often used for length-mass equations (Benke et al. 1999) and preliminary analyses indicated better fits than other model types, e.g. quadratic equations. Residuals of the regression models were tested for correlation with predictor variables.

A.3 Results and discussion

The regression models to predict DM and AFDM from SH and SW were significant (Table A.2). Fits were in excess of 0.8 with the exception of *G. albus*. Fits were generally higher for equations predicting DM than for equations predicting AFDM, as well as higher for equations using SW rather than SH as a predictor. Correlation analyses indicated that there was no significant relationship between predictor variables (SH and SW) and the residuals of the regression models.

The regression models reliably predicted the DM or AFDM of the four snail species across a large size range. SW provided the best predictor of mass, while SH was less accurate. The equations presented in this paper are widely applicable, however a comparison between hard and soft water habitats would be of interest, as this affects shell inorganic composition (Grospietsch et al. 2000, Zettler et al. 2004). Furthermore, information is needed on how size and biomass measurements are affected by preservation time and medium, as can occur in other invertebrate taxa (Edwards et al. 2009).

A.4 References

Benke, A. C., Huryn, A. D., Smock, L. A., et al. (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. **Journal of the North American Benthological Society**, 18(3): 308-343.

Berlow, E. L., Neutel, A. M., Cohen, J. E., et al. (2004) Interaction strengths in food webs: issues and opportunities. **Journal of Animal Ecology**, 73(3): 585-598.

Brose, U., Jonsson, T., Berlow, E. L., et al. (2006) Consumer-resource body-size relationships in natural food webs. **Ecology**, 87(10): 2411-2417.

Burgherr, P. and Meyer, E. I. (1997) Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. **Archiv Fur Hydrobiologie**, 139(1): 101-112.

Cressa, C. (1999) Dry mass estimates of some tropical aquatic insects. **Revista Biologica Tropical**, 47: 133-141.

Edwards, F. K., Lauridsen, R. B., Armand, L., et al. (2009) The relationship between length, mass and preservation time for three species of freshwater leeches (Hirudinea). **Archiv Fur Hydrobiologie**, 173(4): 321-327.

Edwards, F. K., Lauridsen, R. B., Duerdoth, C. P., et al. (2008) Dry and ash-free dry mass to length relationships of bullhead (*Cottus gobio*). **Archiv Fur Hydrobiologie**, 172: 315-316.

Gonzalez, J. M., Basaguren, A. and Pozo, J. (2002) Size-mass relationships of stream invertebrates in a northern Spain stream. **Hydrobiologia**, 489(1-3): 131-137.

Grospietsch, T., Sitnikova, T. Y. and Zerbst-Boroffka, I. (2000) Comparison of calcium storage between a Baikalian gastropod and holarctic relatives. **Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology**, 125(2): 273-283.

Hall, R. O., Wallace, J. B. and Eggert, S. L. (2000) Organic matter flow in stream food webs with reduced detrital resource base. **Ecology**, 81(12): 3445-3463.

Ings, T. C., Montoya, J. M., Bascompte, J., et al. (2009) Ecological networks - beyond food webs. **Journal of Animal Ecology**, 78(1): 253-269.

Johnston, T. A. and Cunjak, R. A. (1999) Dry mass-length relationships for benthic insects: a review with new data from Catamaran Brook, New Brunswick, Canada. **Freshwater Biology**, 41: 653-674.

Kirkegaard, J. (2006) Life history, growth and production of Theodoxus fluviatilis in Lake Esrom, Denmark. **Limnologica**, 36(1): 26-41.
Macan, T. T. (1994) **A key to the British fresh- and brackish-water gastropods with notes on their ecology. Scientific publication No. 13.** Ambleside Freshwater Biological Association.

Mason, C. F. (1977) Populations and production of benthic animals in two contrasting shallow lakes in Norfolk. **Journal of Animal Ecology**, 46: 147-172.

Meyer, E. (1989) The relationship between body length parameters and dry mass in running water invertebrates. **Archiv Fur Hydrobiologie**, 117(2): 191-203.

Sabo, J. L., Bastow, J. L. and Power, M. E. (2002) Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. **Journal of the North American Benthological Society**, 21(2): 336-343.

Smock, L. A. (1980) Relationships between body size and biomass of aquatic insects. **Freshwater Biology**, 10(4): 375-383.

Steele, J. H., Collie, J. S., Bisagni, J. J., et al. (2007) Balancing end-to-end budgets of the Georges Bank ecosystem. **Progress in Oceanography**, 74(4): 423-448.

Towers, D. J., Henderson, I. M. and Veltman, C. J. (1994) Predicting dry-weight of New Zealand aquatic macroinvertebrates from linear dimensions. **New Zealand Journal of Marine and Freshwater Research**, 28(2): 159-166.

Woodward, G., Papantoniou, G., Edwards, F. K., et al. (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. **Oikos**, 117: 683- 692.

Zettler, M. L., Frankowski, J., Bochert, R., et al. (2004) Morphological and ecological features of Theodoxus fluviatilis (Linnaeus, 1758) from Baltic brackish water and German freshwater populations. **Journal of Conchology**, 38: 305-316.

A.5 Tables

Table A. 1 Size range and number (n)of specimens of snails used to derive length-mass equations

	n	Height (mm)		Width (mm)	
		Min	Max	Min	Max
Bithynia tentaculata	32	1.7	8.6	1.6	5.9
Gyraulus albus	30	1.0	1.4	2.1	4.3
Radix balthica	32	1.2	11.1	0.9	8.1
Theodoxus fluviatilis	28	1.7	5.1	2.2	7.3

Table A. 2 Parameters of snail size-mass equations: X = predictor, Y = response, SH = shell height, SW = shell width, DM = dry mass, AFDM = ash free dry mass, b slope, $Log_{10}(a)$ intercept, and R^2 = fit of regression model. All regression models were highly significant ($p < 0.001$).

Taxon	X	Y	b	$Log_{10}(a)$	R^2
Bithynia tentaculata	SH	DM	2.517	-3.792	0.95
	SH	AFDM	2.114	-4.128	0.86
	SW	DM	3.103	-3.801	0.95
	SW	AFDM	2.658	-4.156	0.91
Gyraulus albus	SH	DM	4.161	-2.978	0.79
	SH	AFDM	4.287	-3.580	0.65
	SW	DM	2.206	-3.733	0.89
	SW	AFDM	2.129	-4.289	0.65
Radix balthica	SH	DM	2.559	-4.006	0.93
	SH	AFDM	2.281	-4.427	0.88
	SW	DM	2.605	-3.611	0.95
	SW	AFDM	2.325	-4.077	0.90
Theodoxus fluviatilis	SH	DM	3.520	-3.781	0.98
	SH	AFDM	3.810	-4.743	0.95
	SW	DM	3.011	-3.916	0.99
	SW	AFDM	3.270	-4.917	0.97